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EFFECTS OF THE PHYSICAL ENVIRONMENT ON THE PHYSICO-CHEMICAL PROPERTIES OF PLANT SAPS, AND THE RELATION OF THESE PROPERTIES TO LEAF TEMPERATURE[1]

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(with seven figures)

I. Introduction

The importance of studies on the physico-chemical properties of expressed plant saps, as indices of physiological and ecological relationships, has long been recognized. Such studies have been confined for the most part to the measurement of osmotic pressure, electrical conductivity, and more recently, the percentage of bound water in the expressed sap.

This investigation deals with certain physico-chemical properties of expressed plant saps as influenced by age of plant, soil moisture, and the concentration of salts in the soil. The relationship between osmotic pressure of the cell sap and leaf temperature was also studied.

II. Literature review

That physico-chemical properties of cell sap in plants are receiving much attention among scientific investigators is evidenced by the volume of literature appearing on this subject. Among the contributions are some excellent and comprehensive reviews (**20, 35, 41, 45**). It will therefore be unnecessary here to do more than develop a general view-point of the problem, including only those aspects which have been touched upon in this investigation.

Meyer (**41**), Gortner (**20**), and Sayre and Morris (**53**) have given a comprehensive review of the methods employed in the extraction of plant saps.

Harris and co-workers (**26, 20**) and many others have made extensive use of the method of depression of the freezing point in studies of plant

[1]This investigation was begun at the University of Illinois, and continued at Duke University.

distribution, development, and their relationships to the physical environment. KORSTIAN (**35**), REED (**51**), GAIL (**17**), HARRIS and co-workers (**20**), and others have shown that soil moisture has an obvious effect upon cell-sap concentration. The addition of water to the soil usually diminished the concentration of the plant sap.

HARRIS and co-workers (**25, 26**), WALTER (**55**), CAVARA (**6**), and others have studied the effect of salt concentration in the soil upon the physico-chemical properties of the plant sap. CAVARA reported cryoscopic determinations on saps of high concentration, and in 1905 gave results in full for a considerable series of determinations. His maximum values were found in the sap of halophytes which grew in localities where the concentration of the soil solution increased with the advance of the season. He found freezing-point depressions of 7.25° to 8.50° in *Halocnemum strobilaceum*, and 7.48° in *Salicornia fruticosa*. His determinations were made on sap extracted without previous treatments of freezing, which is necessary to render the tissues permeable, and which has been shown to be essential by DIXON and ATKINS (**9**) and others. ATKINS has already pointed out that these are probably submaximum, because of incomplete extraction.

In 1920, HARRIS and co-workers found in an alkaline area in Utah a plant, *Atriplex nuttallii*, the sap of which had a depression of the freezing-point of 14.4° C., which is equivalent to an osmotic pressure of approximately 173 atmospheres. According to GORTNER (**20**), HARRIS observed a slightly greater depression than this in the sap of *A. confertifolia*. It is obvious that the salt concentration of soil in which plants naturally grow is of great physiological and ecological importance.

A number of investigators have shown that the concentration of tissue fluids of a given species is not constant at all times, and that there exists a gradient within the shoot of an individual plant. Probably the first to give exact data on this phenomenon were DIXON and ATKINS (**9**). While working with untreated leaves of *Fraxinus excelsior*, *Vitis veitchii*, and *Magnolia acuminata*, they found a slightly greater depression of the freezing-point for sap from the more distantly placed leaves. However, the osmotic pressure of the sap from leaves at the same level, but at different times and under different conditions, did not correspond by any means; yet it was usually higher than that of the leaves at a lower level. HARRIS and co-workers (**24**) used the method of pre-freezing in ice-salt mixture. Their results on the depression of the freezing point are similar to those of DIXON and ATKINS, but they found that the relative concentration of electrolytes decreased from the lower to the higher levels, which they believe is due to increased photosynthesis in the upper regions of the tree rather than to the concentration of salts from the soil solution by increased transpiration.

Other workers have observed higher concentrations in the sap of leaves or stems near the apex. REED (**51**) found that the concentration of the sap in the apical portion of a stem was greater than that in the basal region. His data show a greater tendency toward fluctuation in the stem than in the leaf. HURD-KARRER (**30**) states that the specific gravity of the sap extracted from the internodes of corn stalks was higher toward the top of the plant. FERNALD (**15**), working with *Philadelphus* and privet, noted irregularities for the cell-sap concentration of leaves toward the tip of branches. In two cases a marked drop in the sap concentration of the uppermost leaves was shown. KORSTIAN (**35**) reported the sap concentrations to be higher for leaves which were at greater distances from the water supply. PRINGSHEIM (**50**) observed in succulents that the concentration was greater in the upper leaves. He accounted for the internal translocation of water from lower to upper leaves during wilting by the difference in osmotic pressure. BECK (**3**) recently used the same method as employed by PRINGSHEIM, and states, "even though it seems evident that the water passes from the older parts to the younger parts, PRINGSHEIM's explanation is not at all convincing. He did not have a clear conception of the relation between the direction of flow and the osmotic quantities of the cells. His explanation was based on the assumption that the water must flow in the direction of the 0_g gradient, which is not true. The gradient itself which he claimed to exist, is doubtful (**9**, p. 102). (It should be remembered in this connection that PRINGSHEIM employed KNO_3 as plasmolyzing agent, which is unreliable, *cf.* **3**, p. 431.") ZALENSKI (**57**), dealing with other peculiarities of the upper leaves of shoots, also pointed out differences of osmotic pressure. ILJIN and SABOLEVA (**32**) reported similar findings, and in addition found a greater osmotic value in the distal portions of the leaf blade as compared with the basal portions. It must be borne in mind that the last three investigators used the plasmolytic method of determining the cell-sap concentrations. EATON (**12**), working with cotton leaves, reported that the freezing-point depression of the extracted sap from the leaves decreased from 1.57° C. for leaves 74 days old to 1.10° for leaves which were 10 days old. Finally, MARTIN, HARRIS, and JONES (**39**) found the freezing-point depression to be greatest in the juices of the terminal leaf of sorghums, with a progressive decrease in the leaf juices from the top of the plant downward. Also the freezing-point depression of the juices of different internodes was about the same in the flowering stage but increased from the top down to the fourth internode when the plant approached maturity.

The earlier literature in regard to the temperature of leaves has been thoroughly reviewed by EHLERS (**14**), and will be mentioned in this paper only in the discussion of the various phases of the experiments. MILLER and SAUNDERS (**42**) and EATON (**12**) reported that young leaves were cooler

than old leaves, and that the temperature was higher for wilted than for turgid leaves. These relationships are in general keeping with the observations of KIESSELBACH (**33**), LOFTFIELD (**38**), and CLUM (**8**), who used various methods to arrive at their comparisons.

An examination of the literature dealing with the transpiration rate of leaves of different ages shows that investigators, using a number of methods, have found great variability in the directions which the gradient takes in different plants.

ALEXANDROV (**1**), working with the sunflower, found that the upper leaves transpire much more vigorously than the lower ones. ZALENSKI (**58**) carried out experiments on the transpiration of different leaves on the same plant and drew conclusions similar to those of ALEXANDROV. SCHECHNER (BURGERSTEIN **5**) reported that the transpiration of young leaves of many plants was greater than that of older leaves, and that in some other plants the leaves of intermediate age had lower transpiration rates than either older or younger leaves. On the contrary, OVERTON (**46**), while working with *Cyperus papyrus,* found that the older leaves transpire more vigorously than the younger ones. SEELIGER (BURGERSTEIN **5**), working with cut leaves from woody plants, and FLEISCHMANN and HIRZEL (BURGERSTEIN **5**), studying the leaves of hop, drew conclusions very similar to those of OVERTON. GIDDINGS (**19**) found that both the higher and lower leaves of *Silphium laciniatum* transpired less rapidly than did the leaves borne near the middle of the plant. KOKETSU (**34**) reported that the stomatal transpiring power of *Coleus* leaves was greatest at the height of their development. He used the hygrometric paper method for his studies. PALLADIN (**47**) states that the transpiring rate is highest during the period of the greatest activity of the leaf, that is, while it is still growing. He assumes this to be due to both cuticular and stomatal transpiration. Recently EATON (**12**) found that young leaves of cotton were cooler than old leaves. The temperature of the leaves 82 days old was 2.3° C. below that of the air, whereas the temperature of the leaves 26 and 20 days old was 4.1° below that of the air. He found that the temperature of very young leaves, 3 days old, was approximately the same as that of leaves 82 days old.

III. Methods and apparatus

The plants used in this investigation were: five varieties of soy beans (*Soja max*), Illini, Manchu, Ebony, Harbinsoy, and Mandarin, respectively; sunflower (*Helianthus annuus*), milo (*Holcus sorghum* var. *durra*), and cabbage (*Brassica oleracea* var. *capitate,* four varieties). The soy beans were secured from the Department of Plant Breeding at the University of Illinois. The seeds were inoculated by means of soy bean nodules, secured from a field in which this plant had formerly grown.

These plants were selected for various reasons: (1) All of them, with the exception of cabbage, have a rather shallow and limited root system, which probably renders them sensitive to variations in moisture conditions. (2) All of them are commonly grown as annuals in various states. The sunflower is recognized as being genetically a mixture, but data presented later will show that the results are not greatly variable even for such a plant.

The plants were grown in the greenhouse under different moisture conditions and soil salt concentrations. The experiments were performed upon normal plants grown in pots, 4 to 8 inches in diameter. The soil used in the containers was a sandy loam in good tilth, having a field-carrying capacity of 35.10, a moisture equivalent of 9.71, and a wilting coefficient of 4.86. A sufficient quantity of soil was prepared at the beginning of the experiments so as to use but one type of soil and to use it only once. The soil was sterilized and thoroughly mixed before it was used. Soil samples were taken, by means of a special cork-borer, from time to time from the various pots, to determine an average soil moisture throughout the growth of the plants. The water content was then determined. These water-content values are expressed as percentages of the dry weight of the soil. The soil in the containers was kept at a moisture content of approximately the given percentage desired, by the frequent addition of water to replace that lost by evaporation. Frequent additions of water were made by the use of a series of auto-irrigators, designed by Professor CHAS. F. HOTTES, but modified to adapt it to this investigation. Neither the auto-irrigator nor the modifications mentioned will be described here since Professor HOTTES contemplates doing further work with it before publication of his results. The field-carrying capacity, the moisture equivalent, and wilting coefficient were determined by the method described by BRIGGS and SHANTZ (**4**).

The leaf and stem material used was collected from the respective parts of the plant, and placed as rapidly as picked into large test-tubes, 1.75 inches in diameter and 7 inches in length. Tubes of this size accommodate from 50 to 60 gm. of plant parts. Each leaf sample was then weighed, and in all cases, except where different methods of treating the tissue before applying pressure were employed, the tubes containing the plant parts were plunged at once into a freezing box of "dry ice." The freezing box with the samples was set in a refrigerator for the period of freezing, which was always 10–12 hours. Leaves and stems treated in this manner are still frozen when the tubes are unstoppered for extraction of the tissue fluids. All leaf samples were collected between 3:30 and 4:00 o'clock in the afternoon, except where otherwise stated, and determinations were made upon each set within one to two hours after they had been extracted.

Different methods of treating the tissue, before applying pressure, were tried out. Pre-freezing with dry ice proved to be the most satisfactory for this investigation. The amount of sap obtained from a sample depends upon its size, the age and kind of tissue, water content, and on its treatment.

A small but powerful hydraulic press, equipped with a test cylinder 1.12 inches in diameter, with necessary filters and accessories, was used for obtaining the sap samples. This is the standard press, LPI, with 1.12-inch diameter test cylinder and filter pads and pan (S–1722), sold by Fred S. Carver, New York. The pressure was sufficient to leave the press cake relatively dry.

A uniform time of draining and a uniform pressure were used. The following procedure served as a standard: a 50-gm. sample of tissue was subjected to 5000 pounds per square inch, drained for four minutes after maximum pressure was reached, and the volume of sap extracted was measured. These samples were tightly stoppered and placed in ice-water pending determination of the freezing point.

Estimation of dry substance in plant sap by refractometer

The water content of the tissue was obtained from a duplicate sap sample and the water left in the press cake. The dry substance content of the sap was obtained by the refractometer method of Gortner and Hoffman (**21**). An Abbé refractometer (Zeiss) was used.[2] Instead of using the sugar scale for the dry-matter reading, the dry substance was obtained from Geerlig's table used in determining the dry substance in sugar house products (**2**). The percentage of water in the sap was obtained from published tables of the United States Bureau of Standards (**49**). This method was checked by drying a known quantity of sap in the oven at a temperature of 65°–70° C. for a period of 196 hours.

Determination of osmotic pressure of extracted leaf saps

The freezing point of the sap was determined in the usual freezing-point apparatus with the use of a Beckmann thermometer certified by the Bureau of Standards. At least two determinations were made upon each sample, and the average of closely agreeing duplicates was taken as the freezing point. The observed freezing-point depressions were corrected for undercooling by the table of Harris (**22**). The corrected freezing-point depression readings were converted into equivalent osmotic values from Harris and Gortner's tables (**23**).

[2] Lent for the investigation by courtesy of the Department of Chemistry of Duke University.

Determination of bound water

The method employed in this investigation was essentially that introduced by Newton and Gortner (43) but with slight modifications in order to improve the accuracy of the technique. The procedure, as modified, is as follows: The dry substance content is obtained by a refractometer. Then the freezing-point depression of the fresh juice is obtained by the Beckmann method, making the necessary correction for undercooling. Next a fresh sample of juice containing exactly 20 gm. of water is weighed out into a 100-cc. Erlenmeyer flask; this is a convenient quantity for a duplicate determination. Since the principle of the method depends upon the determination of small differences in the amount of free water in the juice, it is important that the juice be accurately weighed. The weight of juice required to contain exactly 20 gm. of water can readily be calculated, after knowing the dry substance content as indicated by the refractometer. To the accurately weighed portion of juice are added 6.8448 gm. of finely pulverized sucrose; this is just sufficient quantity to make a molar concentration in the total water percentage. The sucrose samples were previously weighed out into small porcelain dishes and stored in a desiccator until required for use. After the sucrose has been added to the flask containing the juice it is stoppered and placed in a bath of ice slush and gently moved back and forth in a horizontal motion. Care is used to avoid frothing, which would probably bring about physical changes in the colloids. The time required for the sucrose to dissolve in the juice was found to be 20 to 25 minutes, when the sucrose was finely pulverized.

After the addition and solution of the sucrose, the freezing-point depression of the juice sample is redetermined. This value is usually found to be greater than the sum of the original freezing-point depression of the juice and the sugar solution determined separately. This excess depression is considered to be due to the fact that part of the water is being held as imbibed water by the hydrophilic colloids in the juice and is thus unavailable as a solvent. The molecular concentration of the sugar will therefore be increased more than the theoretical amount; hence an excess depression of the freezing point. The magnitude of the excess depression is taken as a measure of the water held in such a way as not to be available for the solution of the sucrose. The percentage of "bound water" is in turn taken as an index of the relative colloidal content of the plant juice.

The possible sources of error in this method have been discussed by its originators. However, there are certain precautions to keep in mind. The same portion of juice should never be frozen twice, since the frost precipitation of proteins alters the hydration and consequently invalidates the results. For this reason, when bound-water studies were being made, the

plant tissues used were finely ground in a food chopper rather than prefrozen. It is likewise necessary to keep dry the neck and upper wall of the flask. Again, the validity of the method as a measure of the hydrophilic colloid content of a sap rests upon the assumption that there are no other substances present in the juice which bind appreciable amounts of water. Probably the one non-colloidal substance which is likely to be present in plant juices in sufficient quantity to bind appreciable amounts of water is sucrose. This fact should be borne in mind when interpreting the results of an investigation upon different plant saps and the same plant sap when collected under different environmental conditions.

The percentage of bound water is calculated as follows (symbols and formula taken from NEWTON and MARTIN, **45**):

Δ = freezing-point depression of the plant juice.
Δa = freezing-point depression of the juice after a molar concentration has been added.
$\Delta s = \Delta a - \Delta$, the additional depression due to the added sugar.
$\Delta x = \Delta s$ minus the freezing-point depression (determined experimentally) of a molar solution of the sugar used.

$$\text{Bound water} = \frac{\Delta x \cdot 89.2}{\Delta s},$$

where 89.2 is the percentage of free water in a weight molecular solution of sucrose, assuming the formation of sucrose hexahydrate, as explained by NEWTON and GORTNER (**43**).

LEAF TEMPERATURE MEASUREMENTS

No procedure for the direct determination of the individual transpiration rates of attached leaves under experimental conditions has yet been devised, except for the method employed by ALEXANDROV (**1**). His method consisted of wrapping and unwrapping, in turn, the leaves of the upper and lower zones respectively; thus he was able to compare the intensity of transpiration of the two sets of leaves without detaching any of them, and at the same time to employ that most satisfactory of all methods, the weighing of intact, rooted plants. The thermo-electric method was used in this investigation to obtain the leaf temperature. The leaf-temperature method is not only convenient and applicable to attached leaves, but it has a further advantage in that it is rapid. The leaves until the moment of a measurement are fully exposed to a standard light, and during the few seconds required for the reading there is little opportunity for a material change in leaf temperature. Several investigators have found a high correlation between transpiration rates and leaf temperatures; therefore it is possible to use this method to secure the measurements and comparisons.

The thermo-electric method described by EATON and BELDEN (13) is generally followed. The wall galvanometer[3] used was provided with a telescope and scale, and was formerly listed by Leeds and Northrup as no. 2239–d, type P. When purchased it had a coil resistance of 2000 ohms, a sensitivity of 0.002 microcoulomb per millimeter on the scale, and a period of 26 seconds. As wired for use, it had a sensitivity of 0.1204° C. per millimeter. The thermocouples were of no. 36 copper and constantan wire with soldered junctions. They were inclosed in a piece of rubber tubing and attached to a wooden handle as described by EATON and BELDEN.

The galvanometer and thermocouples were standardized by passing a known current through them and by immersing the respective junctions in a series of water baths of different temperatures. In preparation for taking the readings, the scale zero was brought to the cross hair, while the air junction was protected from the light by its paper cone and the leaf junction by the shadow of another piece of paper.

For a short time the thermo-electric apparatus was placed in the greenhouse near the experimental plants; but because relatively large fluctuations in direct sunlight were found, especially when the temperature was high and when the air was in motion even during a brief period of a few seconds, the apparatus was removed to a room where the temperature, humidity, and air currents were nearly constant, at least over a period of several seconds and even minutes. A standard light (standard candle power 61.893) at a distance of three feet was used under these conditions. Most of the temperature determinations were made by two persons. One clasped the leaf surfaces over the thermo-junction while the other recorded the swing of the galvanometer when it came to rest. This procedure permitted fairly rapid work.

Six potted plants, growing under uniform conditions of moisture, etc., were selected at random for the leaf-temperature studies. Three temperature measurements were made on each of the leaves of six plants. Beginning with the lowest leaves, which did not show any signs of deterioration, the temperature of each leaf was measured, proceeding from the older to the younger leaves in turn, while the plant was turned whenever it was necessary to get equal exposure to the light. As soon as the leaf-temperature measurements were completed, these leaves, with similar ones from other plants grown under like conditions, were gathered for the determination of the tissue fluids. The preceding method provided 36 readings for each temperature mean. The mean temperature of the leaves in each of these groups was averaged for the comparison. The probable errors of the means were calculated by the usual formula, since all the determina-

[3] Lent for this investigation by courtesy of the Physics Department of Duke University.

tions exceeded twenty in number. The leaf temperatures were made on leaves, numbers 60–72 and in some cases 72–84, but owing to the small size of leaves numbering 72–84 it was deemed not feasible to collect them for sap analyses.

IV. Experimental data and discussion

Effect of different methods of treating tissue previous to extraction

A comparative study was made of several methods of treating leaf tissues, to determine the effect upon the volume of sap which could be extracted from them under pressure, and the osmotic concentration of this extracted juice. Parallel series of determinations were made on leaves of plants grown in soil containing a very low supply of available water and on others grown with approximately the optimum supply of soil moisture. The leaves were equally divided into four lots and received the following treatments. The leaves of lot no. 1 were placed under a pressure of 5000 pounds per sq. in. without being given any previous treatment; those of lot no. 2 were pre-frozen with solid CO_2; those of lot no. 3 were pre-frozen with an ice-salt mixture; and those of lot no. 4 were previously ground into shredded pulp by means of a food grinder, before extraction of sap by means of pressure.

TABLE I

Effect of different treatments of soy bean leaves upon extractable amount of sap and upon osmotic concentration of extracted sap

Pressure, 5000 pounds per sq. inch; time of draining, 4 minutes

Date (1930 Oct.)	Method of treatment	Moisture content of tissue	Vol. of sap per 100 gm. tissue	Mean age of leaves	Total water extracted	Osmotic pressure in atmospheres	Soil moisture (dry weight)
		per cent.	*cc.*	*days*	*per cent.*		*per cent.*
28	Solid CO_2	(85.3)*	(60.3)	(24)	(70.6)	(9.178)	(17.0)
31	Solid CO_2	79.8	53.6	25	67.2	10.079	9.0
28	Ice-salt mixture	(85.3)	(53.4)	(24)	(62.6)	(9.105)	(17.0)
31	Ice-salt mixture	79.8	37.5	25	47.5	9.154	9.0
28	Ground	(85.3)	(52.2)	(24)	(61.2)	(9.045)	(17.0)
31	Ground	79.8	41.3	25	51.9	10.156	9.0
28	Untreated	(85.3)	(31.6)	(24)	(37.1)	(7.361)	(17.0)

* Brackets indicate that plants were grown in soil containing approximately optimum soil moisture.

Table I records the results of a representative series of determinations upon the leaves of soy bean, variety Manchu. The water content of the leaves was 5.5 per cent. higher in the plants grown under the more favorable water supply, a fact which may be expected to have some influence on the volume of tissue fluids which could be extracted. For plants grown under more favorable conditions of soil moisture, approximately the same volume of tissue fluid was extracted from samples frozen with solid carbon dioxide, from samples frozen with ice-salt mixture, and from samples ground into pulp by means of a food grinder. Whereas, for plants grown under less favorable conditions of soil moisture, only with the sample frozen in solid carbon dioxide was a volume of sap secured which compared with the volumes expressed from plants grown under more favorable conditions of soil moisture. The untreated sample gave a smaller volume of plant sap than any of the treated samples.

MEYER's (**41**) work with pine leaves collected during the summer and winter months shows that pre-freezing with solid CO_2 yields the greater volume of sap. Thus his results are substantiated by those of table I.

COMPARISON OF REFRACTOMETER AND OVEN METHOD FOR DETERMINING DRY SUBSTANCES IN PLANT SAPS

In this type of investigation a direct and yet rapid method of determining the content of dry substance in biological fluids must replace the usual method in which materials are desiccated in an oven. GORTNER and HOFFMAN (**21**) proposed the use of the refractometer for this purpose, for the reason that it is more economical of juice, time, and apparatus. It was deemed necessary, however, to compare these two methods to determine the degree of accuracy of the more rapid method.

The determinations on oven-drying were carried out by desiccating 10-gm. samples of juice in porcelain dishes of 4-cm. diameter, in duplicate, in an oven at 65–70° C. for 196 hours. This excessive length of desiccation seemed necessary because of the difficulty with which the last traces of moisture are driven from the gummy residues left on evaporation. Undoubtedly, volatile materials other than water are given off by oven-drying; however, these substances are thought to constitute a very small portion of the dissolved substances and therefore do not introduce a serious error.

The results in table II show that refractometer readings give only 89.75 per cent. of solids found by oven-drying. Therefore a correction of 10.25 per cent. was used in all data for the dry substance when determined by the refractometer method. The data in table II confirm in general those of NEWTON and MARTIN (**45**), which were published just after the writer had concluded his experiments. However, they found it necessary to make a larger correction than these data show. This difference may

TABLE II

DRY SUBSTANCE IN EXTRACTED JUICE AS DETERMINED BY REFRACTOMETER AND OVEN

SPECIES	DATE OF COLLECTION (1930)	DRY SUBSTANCE		PERCENTAGE OF REFRACTOMETRIC READING FOUND BY OVEN-DRYING
		BY OVEN DRYING	BY REFRACTOMETER	
		per cent.	*per cent.*	*per cent.*
Soja max var. mammoth yellow*	Sept. 15	15.45	17.14	90.1
Soja max var. Virginia*	" "	13.99	15.36	91.1
" " " " * +5% sucrose	" "	18.69	20.73	90.2
Helianthus annuus*	" "	11.66	13.06	89.2
Zea mays*	" "	11.41	12.87	88.7
Soja max var. Manchu†	Oct. 14	8.97	10.09	88.8
Soja max var. Illini†	" "	9.27	10.23	90.6
" " " " †	" 22	9.267	10.41	89.02
Soja max var. Manchu†	" "	8.36	9.28	90.0
Average				89.75

* These plants were collected from fields about 3 miles northwest of Durham, N. C.
† These plants were grown in the greenhouse.

be due to the fact that they used a vacuum oven at a temperature of 96°–97° C. for the last 48 hours of desiccation, thereby making it more probable that the last traces of the moisture were driven from the residue. If the correction used in this investigation is too low, the bound-water determinations given later in this paper are in consequence slightly low.

A deviation of 0.2–0.4 per cent. represents the probable error in dry substance shown in the data in table II. This is not of sufficient magnitude to affect the comparative values.

EFFECT OF STORING PLANT SAPS AT 0° AND 20° C.

The tissue fluids were stored for a short time only after being extracted, before the desired determinations were made. The interval between extraction and the making of the determinations seldom exceeded one hour. In this interval the changes in the properties of the tissue fluids should be inappreciable, when stored at 0° C.

In order to ascertain the effect of time and temperature upon the osmotic value, samples of juice were divided and one part was stored at a constant temperature of 0° and the other portion at 20° C. The samples

were held for a maximum period of about 6 hours. At desired intervals portions were withdrawn for the determination of the osmotic value. The results of this experiment are shown in table III.

TABLE III

EFFECT OF DURATION AND TEMPERATURE OF STORAGE ON THE OSMOTIC VALUE OF PLANT JUICES

PLANT JUICE USED	DATE (1930)	TIME OF STORAGE	TEMP. OF STORAGE	DEPRESSION OF FREEZING POINT	OSMOTIC PRESSURE
		hours	°C.	°C.	*atm.*
Soja max var. Manchu, 8.5 per cent. solids	September 30	0.0	0	0.783	9.43
		1.0	0	0.784	9.44
		2.3	0	0.803	9.67
		4.2	0	0.811	9.76
		6.0	0	0.819	9.86
		0.50	20	0.820	9.87
		1.45	20	0.885	10.66
		2.80	20	0.908	10.94
		3.50	20	0.923	11.02
		5.23	20	0.946	11.39
Soja max var. Illini, 9.1 per cent. solids	October 2	0.00	0	0.776	9.35
		1.10	0	0.781	9.41
		2.45	0	0.793	9.55
		4.30	0	0.807	9.62
		6.10	0	0.806	9.61
		1.45	20	0.821	9.89
		3.00	20	0.843	10.16
		4.68	20	0.870	10.48
		6.70	20	0.884	10.65

These results show that the osmotic pressure in plant saps in most cases increased gradually during storage, the greater change occurring at 20° C. This increase in osmotic pressure is to be expected owing probably to hydrolysis of various substances in the juice. The rate of change in osmotic pressure at 0° C. is so slight during the first hour, the time normally required for the routine determinations, that the changes are considered inappreciable.

EFFECT OF MATURITY OF PLANTS ON CONCENTRATION AND OSMOTIC PRESSURE OF JUICES

It was desired to ascertain the effect of maturity of the plant upon the concentration, osmotic pressure, and percentage of moisture within the leaves of two varieties of soy beans and the sunflower.

The percentage of composition at different periods from near the flowering stage to near maturity for *Soja max*, varieties Mandarin and Harbinsoy,

TABLE IV

PERCENTAGE COMPOSITION OF LEAVES AT DIFFERENT PERIODS FROM NEAR FLOWERING STAGE TO APPROACH OF MATURITY

SPECIES	DATE (1930)	AGE OF PLANT	WATER CONTENT OF LEAVES	CONCENTRATION BY REFRACTION	OSMOTIC PRESSURE	REMARKS
		days	*per cent.*	*per cent.*	*atm.*	
Soja max var. Harbinsoy	Jan. 13	35	85.5	7.7	8.583	Soil moisture 17.0 per cent.; few flowers
	Jan. 23	45	79.2	8.9	9.065	Flowers and few pods
	Feb. 4	57	77.5	10.1	10.157	Pods about 2″ long
	Feb. 16	69	75.2	10.8	10.873	Beans in pods of medium size
Soja max. var. Mandarin	Jan. 13	36	81.8	8.6	9.046	Soil moisture 14 per per cent.; flowers and small pods
	Jan. 23	46	78.3	10.4	10.123	Pods 1–1½″ long
	Jan. 28	51	74.4	11.3	10.877	Pods 2″ long; small beans
	Feb. 4	58	75.5	11.0	10.912	Beans of medium size
Helianthus annuus	Jan. 15	35	91.8	5.7	7.631	Soil moisture 17.0 per cent.; no flower buds
	Jan. 28	50	86.2	7.3	8.690	Flower buds
	Feb. 7	60	84.6	8.5	8.946	Flower buds open

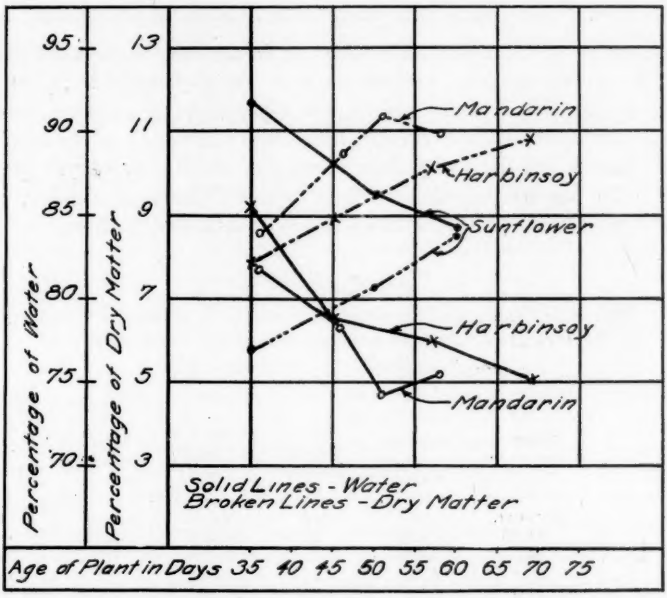

FIG. 1. Percentage of dry matter and water in soy bean and sunflower plants at different periods from near the flowering stage to approach of maturity.

and *Helianthus annuus* is given in table IV and presented graphically in figure 1.

The results in table IV show that with the approach of maturity the moisture content of the leaf tissue decreases rather rapidly, causing corresponding increase in concentration and osmotic pressure of the sap. These data are presented graphically in figure 1. The curves of this figure are of the same general trend; however, in the variety Mandarin the moisture content drops rapidly and the dry substance ascends rapidly. This is probably due to a difference in maturity of the two varieties, since the Mandarin variety bore small pods whereas the Harbinsoy had only flower buds. The sunflower plants had not reached maturity, as can be noted from the table and graph.

WELTON, MORRIS, and HARTZLER (**56**) state that the percentage of residual dry matter increased from the tasseling stage to maturity in both varieties of corn studied. The percentage of moisture decreased in the Clarage variety in the early part of the season, but the trend was reversed at maturity; the percentage of moisture decreased in the Burr Leaming throughout the period studied.

Effect of collecting plant parts at different times

On account of the well known variation in the osmotic pressure of plant saps throughout the day, collections were usually made at 4:00 P. M. However, since this investigation in part deals with the gradient of osmotic value of leaves, their water content and temperature, it was deemed necessary to make a few determinations at another hour of the day. Likewise, Eaton (**12**) has recently drawn more or less definite conclusions on the gradient within the plant on data secured from one experiment on cotton leaves.

TABLE V

Ages, water content, and freezing-point depressions of extracted tissue fluids of leaves at successively higher nodes on main stem of soy bean var. Manchu; November 6, 1930

Time	Moisture content	Vol. of sap per 100 gm. of leaves*	Mean age of leaves	Freezing-pt. depression	Osmotic pressure	Total water extracted
	per cent.	*cc.*	*days*	(Δ)	*atm.*	*per cent.*
10:30 A. M.	84.05	54.98	38	0.553	6.663	65.4
	84.0	57.96	33	0.608	7.325	69.0
	83.8	57.4	26	0.631	7.602	68.4
	81.9	61.3	18	0.662	7.975	74.8
	82.01	55.8	13	0.776	9.346	68.03
	Lowest ⅓ of stem			0.631	7.602	.....
	Middle " " "			0.658	7.962	.....
	Upper " " "			0.738	8.889	.....
4:00 P. M.	82.5	.....	38	0.741	8.925	.....
	82.4	.....	33	0.748	8.999	.....
	81.9	.....	26	0.667	8.035	.....
	80.7	.....	18	0.664	7.999	.....
	80.6	.....	13	0.642	7.734	.....
	Lowest ⅓ of stem			0.776	9.346	.....
	Middle " " "			0.737	8.877	.....
	Upper " " "			0.673	8.107	.....

* By doubling volumes obtained experimentally from 50-gm. sample.

The plants used for the data in table V were grown at a constant soil moisture of 15.6 per cent. The data show that if collections are made about 10:30 A. M., results will be secured which differ considerably from those obtained at 4:00 P. M. The osmotic concentration for the successively higher nodes on the main stem are just the reverse, and the segments of the stem show the same general trend as that of the leaves.

Harris, Gortner, and Lawrence (**24**), in a study of the relation between the osmotic concentration of leaf sap and the height of leaf insertion in trees, noted that the osmotic pressure increased with the height at which

the leaves were collected, whereas the specific electrical conductivity decreased from the lower to the higher levels. According to these data, evidently there is an increased photosynthesis in those leaves in the upper portion of a tree, as indicated by a decreased ratio of $\frac{K}{\Delta}$ and an increased osmotic pressure. This being the case, one would expect results similar to those in table V, on account of the ratio of photosynthesis to translocation of the food materials during the period extending from late afternoon throughout the night. Whether this increase in photosynthesis in the upper and younger leaves is due to more favorable light conditions or to a more favorable ratio of the electrolytes present in the cell sap can be determined only by more extensive studies.

Temperature fluctuation in experimental room

The degree of fluctuation of temperature within the room in which the thermo-electrical apparatus was located was observed for a period of six hours; during this period the temperature fluctuated to the extent of $\pm 0.2°$ C. Since the variability in temperature in this room was found to be slight and did not change at a very rapid rate, it is not thought to introduce appreciable error into the leaf-temperature determinations, to be presented later. Nevertheless an average of a number of consecutive determinations was made in order that a more exact index of the temperature changes of leaves and of the surrounding air might be obtained. Each temperature mean is the average of from 20 to 36 determinations taken during a 20- to 30-minute period. It must be borne in mind, in making leaf-temperature determinations, that they are relative only to the conditions that prevail when the determinations are made. This is due to the fact that the temperature of a leaf is influenced by such factors as temperature of the air, available soil moisture, air currents, age and type of leaf, and intensity of the light exposure.

Gradient within the plant

The leaf temperature measurements were made and the leaves collected for extraction of the tissue fluids on October 30, 1930, for the data in table VI. The temperature measurements were started at 2:00 and ended at 2:39 P. M. The leaves were collected and frozen at 4:00 P. M. The air temperature at 2:00 was 74° F. and the wet bulb was 64° F.; thus the relative humidity was 58 per cent. and the dew point 64° F. The average soil temperature at the same time was 68° F.

The leaves are grouped according to both age and position on the plant. The ages designated are the means of the leaves in each group. All of the plants used were of about the same height, and the leaves rarely differed in age from the mean more than two days.

TABLE VI

SUMMARY OF PHYSICAL PROPERTIES OF SOY BEAN LEAVES, VAR. MANCHU, AND LEAF TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 30, 1930
(SOIL MOISTURE 15.0 PER CENT.; WEATHER CLEAR AND SUNNY)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	*° C.*	*° C.*	*atm.*	*per cent.*
1–24	85.64	30	56.4	−0.42 ± .04	0.964	11.608	66.1
24–36	85.76	25	67.1	−0.68 ± .06	0.762	9.178	78.2
36–48	86.60	18	68.6	−0.72 ± .05	0.756	9.105	79.7
48–60	83.60	10	67.7	−0.97 ± .08	0.725	8.732	80.4
60–72	81.24	4	.....	−0.78 ± .07	.....	.....	.....

* By doubling volume obtained experimentally from 50-gm. sample.

Table VII records the results obtained from leaves of variety Manchu, 38 days old, grown at a soil moisture of 9.5 per cent. The leaf-temperature measurements were started at 2:30 and ended at 3:12 P. M. The air temperature at 2:30 P. M. was 72° F. and the wet bulb was 59° F.; thus the relative humidity was 65 per cent. These plants were exposed to sunshine during the morning but it was cloudy during the afternoon.

Table VIII presents data on *Helianthus annuus* grown at a soil moisture of 15 per cent. The leaf-temperature measurements were carried out be-

TABLE VII

SUMMARY OF PHYSICAL PROPERTIES OF SOY BEAN LEAVES, VAR. MANCHU, AND LEAF TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 31, 1930
(COLLECTED AT 4:00 P. M.)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	*° C.*	*° C.*	*atm.*	*per cent.*
1–24	81.3	30	43.0	+1.03 ± .03	0.837	10.079	52.9
24–36	79.8	26	53.6	+0.91 ± .05	0.828	9.971	67.1
36–48	79.4	17	59.7	+0.86 ± .03	0.789	9.502	75.1
48–60	78.1	10	59.1	+0.64 ± .04	0.778	9.370	75.6
60–72	79.7	4	.....	+0.68 ± .02	.....	.....	.....

* By doubling volume obtained experimentally from 50-gm. sample.

tween 3:00 and 3:40 P. M. The relative humidity in the experimental room was 60 per cent. The soil temperature was 69° F. The leaves were collected for freezing and storage at 4:00 P. M.

TABLE VIII

SUMMARY OF THE PHYSICAL PROPERTIES OF SUNFLOWER LEAVES AND TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 31, 1930
(CLEAR AND SUNSHINY DAY)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	*° C.*	*° C.*	*atm.*	*per cent.*
1–24	90.0	28	61.3	−0.81 + .05	0.754	9.081	68.8
24–36	91.3	20	64.1	−0.89 − .03	0.725	8.732	70.2
36–48	86.4	14	62.0	−0.97 − .02	0.724	8.720	71.7
48–60	87.7	10	64.0	−0.96 − .04	0.726	8.744	72.9
60–72	85.0	6	.....	−0.93 ± .03	.....	.....	.....

* By doubling volume obtained experimentally from 50-gm. sample.

The results in tables VI, VII, and VIII are shown graphically in figures 2, 3, and 4 respectively.

The leaves were found to be progressively cooler from the older, upward, toward the younger, until leaves a few days old were reached. This was observed to be true whether the leaf-temperature determinations were made at 10:30 A. M. or at 4:00 P. M. For soy bean plants grown at a soil moisture of 15 per cent. of the dry weight, the oldest leaves had the highest temperatures, −0.42° C. below the air temperature. Leaves 18 and 10 days old were the coolest, −0.72° and −0.97° C. respectively, below the air temperature. Leaves 4 days old were warmer than those 10 days old, and had a temperature −0.78° C. below that of the air. Whether the increased transpiration rate, as determined by the leaf-temperature determinations, of the leaves 10 and 18 days of age is the result of both stomatal and cuticular transpiration can be determined only by more extensive studies. Likewise it has been reported by a number of investigators that there is an increased respiration rate in the young developing organs, which fact might therefore aid in the explanation of why leaves 4 days of age were warmer than those 10 days old.

The oldest leaves of soy bean plants grown in a soil which had a moisture content of 9.5 per cent. of the dry weight had the highest temperatures. However, instead of being below the air temperature, in all age groups their temperature was above that of the air.

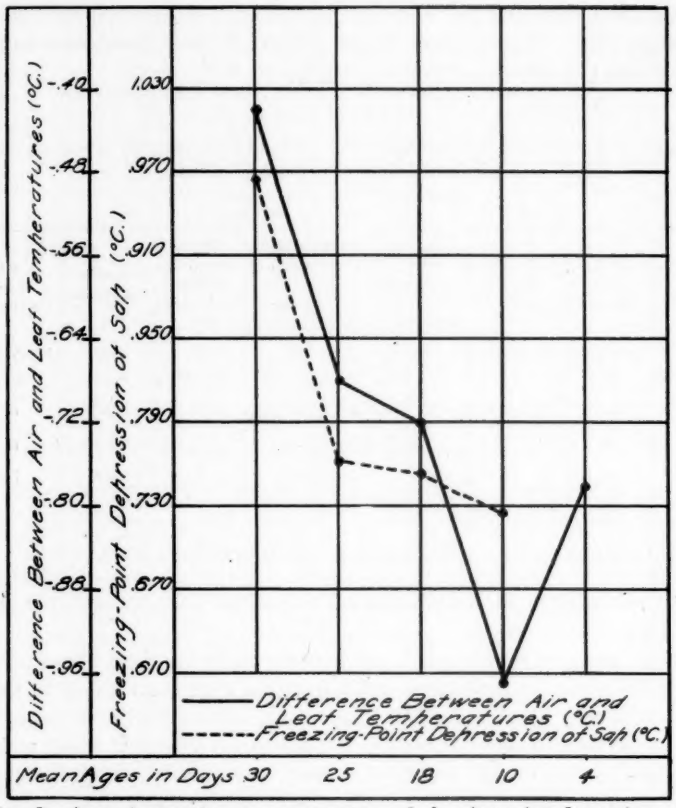

FIG. 2. Ages, temperature measurements, and freezing-point depressions of extracted sap of leaves at successively higher nodes on stem of soy bean plants (variety Manchu, table VI).

The leaf-temperature determinations for the sunflower plants grown at a soil moisture content of 15 per cent. of the dry weight was in the same general order as those of the soy bean plants grown at this soil moisture.

The freezing-point depression increased with the age of the leaves and decreased with the height of their insertion for the plant portions listed in tables VI, VII, and VIII. However, it is probable that this would have been just the reverse if collections of the leaves had been made at 10:30 A. M. rather than at 4:00 P. M. This conclusion is indicated from data presented in table V.

Considering only the osmotic concentration, the upper leaves would be placed at a disadvantage with respect to older leaves in their ability to with-

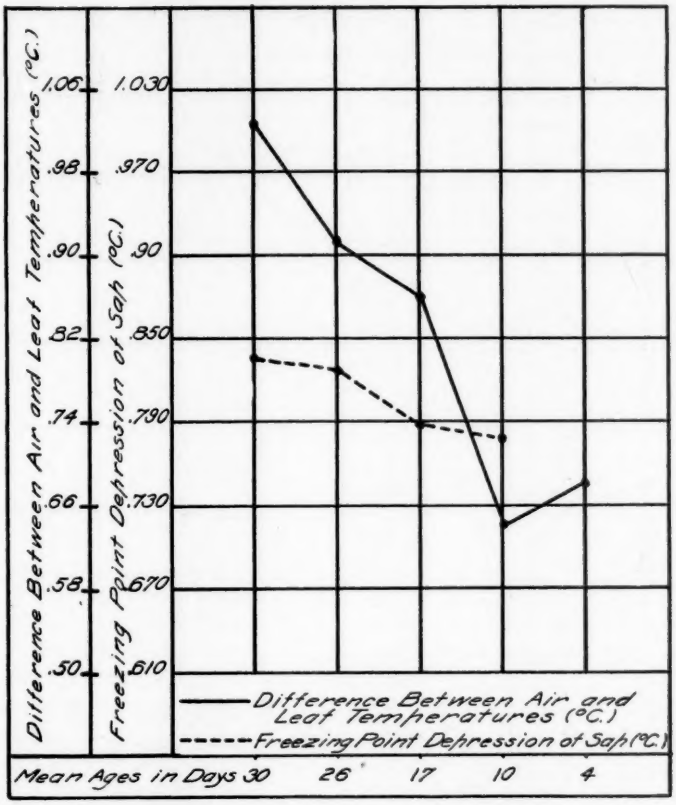

FIG. 3. Ages, temperature measurements, and freezing-point depressions of extracted sap of leaves at successively higher nodes on stem of soy bean plants (variety Manchu, table VII).

draw water from the transpiration stream, at 4:00 P. M. In addition to the difference in osmotic concentration, the upper leaves of soy bean and sunflower were also at a disadvantage in their water relations as a result of their difference in position on the main stem.

It was thought desirable to make a study of the water content of different tiers of leaves of soy bean and sunflower, before wilting and during different degrees of wilting. Table IX presents these data. The plants were grown in pots, and water was withheld to induce wilting. The common method of weighing, drying, and reweighing was employed to determine the water content of leaves. Immediately after picking, the leaves were placed

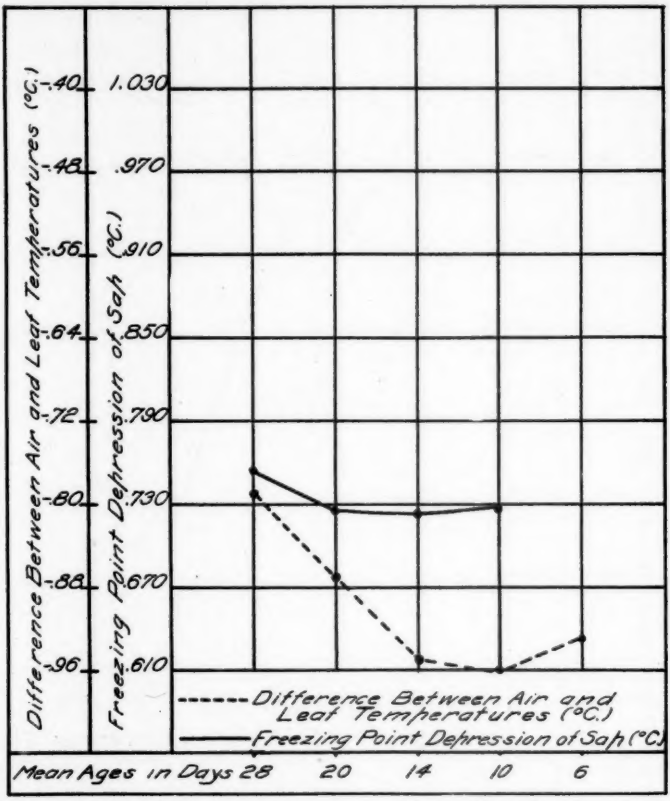

FIG. 4. Ages, temperature measurements, and freezing-point depression of extracted sap of leaves at successively higher nodes on stem of sunflower plants.

in blackened glass tubes which were tightly stoppered. After being weighed the tubes were opened and placed in a drying oven at a temperature of 90°–95° C. The final dry weight was obtained by reweighing the stoppered tubes until they had reached a constant weight.

The results given in table IX show that lower leaves of both soy bean and sunflower have a higher water content than have the upper leaves. Nevertheless the lower leaves either give up some of this water to the upper ones during wilting or it is lost through transpiration, whereas the upper (younger) leaves have some means of retaining their water content.

It may be noted in the case of soy bean (table IX) that the lowest tier of leaves lost 11.75 per cent. of their water content whereas the upper tier lost

TABLE IX

PERCENTAGE OF WATER IN DIFFERENT TIERS OF LEAVES OF SOY BEAN VAR. ILLINI AND SUNFLOWER BEFORE AND AFTER 24 HOURS OF WILTING; TIME OF COLLECTION 3:30 P. M.

DATE	PLANT	TIER OF LEAVES	MOISTURE		WATER LOST
			BEFORE WILTING WAS NOTED	24 HOURS AFTER WILTING WAS NOTED	
			per cent.	*per cent.*	*per cent.*
Nov. 20, 23, 1930	Soy bean	First (lowest)	85.9	75.8	11.76
		Second	85.3	75.1	11.95
		Third	85.3	75.5	11.49
		Fourth	85.2	76.4	10.32
		Fifth	84.1	77.3	8.09
Jan. 21, 23, 1931	Sunflower	First (lowest)	90.3	72.8*	19.38
		Second	90.05	78.6	12.72
		Third	89.5	85.6	4.36
		Fourth	89.08	87.7	2.78
		Fifth	87.5	85.2	2.63
		Sixth	85.07	84.0	1.27

* These leaves had turned slightly yellow near the margin.

only 8.09 per cent. The sunflower plants showed an even greater difference. The lowest tier of leaves lost 19.38 per cent. of their water content, in contrast to 1.27 per cent. for the sixth tier of leaves.

The fact that young leaves retain their turgidity for a longer period than older ones has been explained by PRINGSHEIM (50) and others to be the result of a greater osmotic pressure. According to PRINGSHEIM, not only the earlier wilting but also the complete drying of adult parts of plants may be explained by the comparatively low osmotic pressure in these parts.

It is hardly possible to explain the more rapid giving up of water by the lower leaves exclusively by reduced osmotic pressure; for if the upper leaves had a greater osmotic concentration than the lower, as shown when collections were made at 10:30 A. M., the loss of their water content would bring about a greater concentration within the cells, which would therefore arrest the further loss of water. Moreover, the loss of water would be possible only in the case of simultaneous death of young as well as old organs, which apparently does not occur. However, with due recognition of factors which may lead to shifting of equilibria and which must be constantly operative in physiologically active plant cells, we must assume that the movement of water is due only partly to differences in osmotic pressure of the cells; other important factors operating in this connection are probably imbibition pressure and tensile strength of water.

In any transpiring plant there must be a pressure gradient throughout the cell contents to the outside of the cell wall, as well as from cell to cell. As the water vapor diffuses from the intercellular spaces of the leaf to the external atmosphere, the vapor pressure in these spaces is lowered and the liquid water leaves the film on the neighboring cell walls to aid in the establishment of a new equilibrium between the vapor and liquid phases. Therefore, if the rate of evaporation exceeds that of absorption, as in the case of wilting, water which leaves the cell walls cannot be entirely replaced, and thus the concentration of the cell fluids will be increased. In order to maintain this equilibrium, the water film will recede into the pores of the cell walls, the surface force increasing with curvature of the menisci, until an equilibrium is established between the osmotic and imbibition pressures of the cell fluids. Since the logarithmic nature of an imbibition curve means that in a cell containing a high concentration of colloids the pressure goes up very rapidly, therefore the rate of outward diffusion must be slower than in a cell lacking such protection. Since the upper, therefore younger, leaves contain a high percentage of hydrophilic colloids, it is suggested that this condition will aid greatly in the explanation of why the younger leaves in the experiment of table IX were able to retain a greater percentage of their water content during wilting. NEWTON and MARTIN (**45**) point out a close correlation between hydrophilic colloids and drought resistance.

Again in this connection, HARRIS and GORTNER (**20**) found that *Atriplex nuttallii* has a freezing-point depression of 14.4° C., whereas in cactus it is 0.53° C. It was observed by these investigators that *A. nuttallii* did not retain its water as effectively as did the cactus plant. Since their investigation, NEWTON and MARTIN have shown the cactus to have a high hydrophilic colloid content, as determined by the bound-water method.

The unequal distribution of water in the leaves of plants as shown by this investigation substantiates the findings of KRASNOSSELSKY-MAXIMOV (**36**). She likewise noted the water content to be greater in the lower leaves. Her data show that during the daytime, the excess of transpiration over absorption causes the more vigorous parts of the plant, namely, the upper leaves, to abstract water from the lower, less active leaves. On account of this the upper leaves, although subjected directly to the drying influence of sun and wind, were found to exhibit smaller variations in water content than the lower leaves, which are more or less shaded and nearer to the roots.

EFFECT OF PERIODICAL WILTING ON OSMOTIC PRESSURE

The soy beans (data in table X) were grown at about the optimum soil moisture content for a period; then they were periodically subjected to near the point of permanent wilting, after which the plants were once more watered to bring them back to optimum soil moisture. The plants were sub-

jected to severe wilting six times during development, and collections were made when they were 47 days old.

It may be of interest to state that the interval between wilting and death was observed for soy beans, milo, and cabbage. The death of soy beans occurs a few days after wilting, and after many days, even two or more weeks, for milo and cabbage. The number of days before death occurs depends upon a variety of physical conditions. The milo and cabbage are hardier to drought and cold than the soy bean. MAXIMOV (**40**) noted, in his experiments on wilting of plants in relation to drought resistance, that the more drought-resistant plants were capable of losing 30–40 per cent. of their water content without injury, while the less resistant plants showed a reduction in dry weight and shedding of their leaves.

TABLE X

SUMMARY OF DETERMINATIONS MADE FROM COLLECTIONS AT 4:00 P. M. ON SOY BEAN, SUBJECTED TO PERIODICAL WILTINGS; DECEMBER 2, 1930

MOISTURE CONTENT	VOL. OF SAP PER 100 GM. LEAVES	MEAN AGE OF LEAVES	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WAX EXTRACTED
per cent.	*cc.*	*days*	Δ	*atm.*	*per cent.*
75.4	.....	45	.....	.....	.....
75.1	39.3	38	1.176	14.152	52.2
75.3	33.3	31	1.033	12.436	46.0
76.0	38.4	25	1.046	12.596	50.1
78.7	43.1	18	1.001	12.052	54.7

The plants subjected to periodical wiltings (table X) had a higher osmotic concentration than the check plants (table XI) grown at approximately optimum moisture content. Further, it may be noted that the water content of the leaves was less in the case of the periodically wilted plants. This difference in water content might account for part of the increase in the osmotic concentration of these plants; however, it is not thought to account for the entire difference. In the check plants the lower leaves contained the greater percentage of water, whereas in the periodically wilted plants the upper leaves contained the greater percentage. Periodical wilting tends to induce a more xeromorphic structure, a higher osmotic concentration, and a possibly greater hydrophilic colloid content within the cells of the leaves, which may in turn aid in neutralizing the unfavorable consequences of the initial wilting, in spite of subsequent exposures to deficiency of soil moisture. Assuming this, wilting during the early stages of development may be actually beneficial, especially during some seasons.

TABLE XI

SUMMARY OF DETERMINATIONS MADE FROM COLLECTIONS AT 4:00 P. M. ON SOY BEAN, 47 DAYS OF AGE, GROWN AT NEARLY OPTIMUM SOIL MOISTURE CONTENT; DECEMBER 2, 1930

MOISTURE CONTENT	VOL. OF SAP PER 100 GM. LEAVES	MEAN AGE OF LEAVES	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
per cent.	*cc.*	*days*	Δ	*atm.*	*per cent.*
83.5	51.1	45	0.776	9.346	.....
83.3	52.0	41	0.778	9.370	62.5
81.4	52.2	36	0.776	9.346	64.1
80.7	54.5	29	0.739	8.901	67.5
81.1	60.8	23	0.671	8.083	74.9
80.3	63.3	15	0.658	7.926	78.8

EFFECT OF ADDING NaCl TO SOIL SOLUTION ON OSMOTIC CONCENTRATION OF PLANT SAPS

A number of investigators have shown that sodium chloride added to the soil solution brings about an increase in the osmotic concentration. HARRIS and PASCOE (**26**) state that there is in general a positive correlation in cotton between the salinity of the soil and the osmotic concentration as measured in terms of freezing-point depression. In order to find whether soy bean of Manchu variety behaved in like manner, the following experiment was carried out. Plants were watered with a solution of 0.05 molar

TABLE XII

EFFECT OF ADDING 0.05 MOLAR SOLUTION OF NaCl TO SOIL SOLUTION IN WHICH SOY BEAN PLANTS WERE GROWING; JANUARY 30, 1931

MOISTURE CONTENT	MEAN AGE OF LEAVES	FREEZING-POINT DEPRESSION OF SAP	OSMOTIC PRESSURE
per cent.	*days*	° *C.*	*atm.*
84.2	38 and 35	1.030	12.400
84.3	29 and 22	1.047	12.604
81.1	20 and 10	1.095	13.180
Check plants			
85.1	39 and 36	0.937	11.284
85.0	31 and 25	0.954	11.488
80.3	22 and 14	0.973	11.716

concentration of NaCl, instead of tap water as for the check plants, in order to keep them at the desired soil moisture. The plants were collected at 9:40 A. M. for freezing and storage.

The results (table XII) show that a 0.05 molar solution of NaCl added to the soil solution increases the osmotic concentration in the case of the soy bean, variety Manchu. The percentage increase amounts to 9.68 when all tiers of leaves are considered together.

Effect of varying soil moisture on osmotic pressure of the plant

Soil moisture has repeatedly been observed to have a marked influence on plant growth. When a plant is grown at or near a minimum soil moisture content, or when subjected to periodical wiltings, its anatomical structure and development are profoundly influenced. Leaves of the soy bean when grown under such conditions were about one-third to one-half as large as those grown under the optimum conditions of soil moisture. The leaves were a darker green, had a denser network of veins, and had a greater number of stomata per unit area. According to Maximov (**40**), these structural changes, which may be termed "xeromorphic," tend to increase transpiration and simultaneously to increase the gaseous exchange. He states, "by virtue of this, xeromorphic plants are distinguished not by a lower but by a higher rate of such processes as transpiration and assimilation."

The percentage of soil moisture is given (table XIII) in tenths (for example, as 9.5 instead of 9.0), because soil samples were taken from time to

TABLE XIII

Effect of soil moisture on freezing-point depression, thus osmotic pressure; soy bean plants 38 days old; collected at 4:00 P. M.

Date	Variety	Concentration by refraction	Freezing pt.-depression of sap	Osmotic pressure	Soil moisture
1930		*per cent.*	Δ	*atm.*	*per cent.*
Nov. 4	Manchu	7.4	0.813	9.791	9.5
Nov. 4	Manchu	7.1	0.809	9.743	10.4
Nov. 4	Manchu	6.5	0.801	9.647	11.8
Nov. 5	Illini	5.7	0.711	8.654	13.7
Nov. 4	Manchu	6.8	0.789	9.502	15.0
1931					
Jan. 21	Ebony	5.3	0.746	8.985	15.0
Jan. 21	Ebony	4.5	0.728	8.768	16.2
Jan. 21	Ebony	4.6	0.715	8.612	17.0

time and the percentage of water determined. Therefore when all of the results of the samples were averaged together, it gave 9.5 rather than 9.0 per cent. None of the soil samples taken varied more than ± .9 of 1 per cent. These data show that the osmotic pressure varies with physiological scarcity of water. It is probable that still more marked differences might be obtained by the use of other plants.

The soy bean has not been found to be very drought-hardy. Likewise, these plants were grown under a constant soil moisture and a nearly uniform temperature and humidity, which in all probability would influence the osmotic concentration. As previously pointed out, the subjection of a plant to periodical wiltings increases its osmotic concentration and probably its hardiness against further loss of water. Greater difference in osmotic concentration has been pointed out between the preceding range of soil moistures; however, they were determinations made on plants grown out of doors, where they were subjected to great changes in soil moisture, humidity, and temperature. These changes tend to increase the osmotic concentration.

DRABBLE and DRABBLE (**11**) were apparently the first to investigate the relation between osmotic pressure of the cell sap in plants and soil moisture. They found it to vary directly with the scarcity of water. Thus the present results substantiate their observations as well as those of some later investigators, previously mentioned.

GEBHARDT (**18**) has recently stated that the saps of plants of different systematic and ecological groups grown under the same soil conditions have about the same osmotic pressures. The results of NEWTON and MARTIN (**45**) do not substantiate this statement, and there are no marked indications of this in the present investigation.

VARIETAL DIFFERENCES IN SOY BEANS

In soy beans there is a complete series of varieties ranging from very early to very late maturing ones. Observations have been made as to the comparative behavior of Ebony, Manchu, and Mandarin varieties with respect to hardiness when grown under partially controlled conditions.

The temperature of 75°–76° F. appears to be approximately optimum. All varieties were found to be sensitive to cool temperatures. A few pots of each variety, of the same age and grown under identical conditions, were placed in a lighted room at 38° ± 2° F. for a period of 72 hours, to see whether any difference could be noted in the varieties. They showed about an equal degree of sensitiveness to this temperature; however, Manchu and Mandarin showed a smaller degree of injury than Ebony. SELLSCHOP and SALMON (**54**) found that Virginia soy beans were severely injured by an ex-

posure of 72 hours, whereas Manchu survived an exposure of 120 hours without injury, when subjected to a temperature of 0.5°–5° C.

It was likewise desirable to know whether these varieties differed as to drought hardiness. A number of pots of each variety were planted and kept at constant soil moisture until they had attained a height of about 8 inches; then they were placed upon a table and allowed to wilt severely before adding enough water to bring back the required soil moisture. The plants were permitted to wilt severely at three different times. After the last wilting they were left unwatered for 7 days. At the end of this period the soil in the pots was dry and the plants in a state of permanent wilting, several having perished. Water was again added to the soil, to permit the plants not already dead to recover. After a few days, record was made of those that had died and of those that had survived. It should be stated that severe wilting greatly reduces the yield, the percentage of which, however, was not determined in these plants. The results are given in table XIV.

TABLE XIV

ENDURANCE TO WILTING OF DIFFERENT VARIETIES OF SOY BEAN PLANTS

VARIETY	NUMBER OF PLANTS		SURVIVAL PERCENTAGE
	IN EXPERIMENT	WHICH SURVIVED	
Manchu	24	10	41.7
Ebony	18	6	33.3
Mandarin	21	11	52.4
Harbinsoy	19	8	42.1
Illini	11	5	45.4

The results (table XIV) show no marked difference between the varieties; however, Mandarin showed a slightly higher percentage of survival than any of the others. Since this is an early variety, possibly it has the potentiality of building up substances which aid it better to withstand drought conditions, although this can be determined only by more extensive studies.

In a variety trial on frost resistance at Muskegon, Michigan, in 1909 (**48**), the comparative resistance of varieties was reported as follows, the first being least injured: (1) Ogemaw; (2) Haberlandt, Ito San, Kingston, Guelph; (3) Habaro, Shingto, Manhattan, Brindle; (4) Jet; (5) Meyer, Chernie. Since the Ogemaw is a very early variety and was found to be the most resistant to frost, there may be a correlation between early varieties and resistance to cold and drought. Maturity of the plant is probably an important factor in either case, and one might expect the same variety to vary in cold and drought resistance, depending upon its stage of maturity.

Only slight variations of the osmotic concentration have been observed for the different varieties when grown under as nearly identical conditions as possible. This is probably to be expected since osmotic values have been found to be largely the result of the environment. However, it is not intended to discount the importance of osmotic properties in the water relations of plants. This point will be touched upon again in connection with the bound-water studies.

V. Bound-water studies in cabbage and milo

There are undoubtedly many factors concerned in the ability of plants to survive a drought or a cold period; but, above all, the potentiality of a plant to build up hydrophilic colloids seems to be of the utmost importance. NEWTON and MARTIN (**45**) have recently pointed out the importance of these substances in relation to drought resistance. ROSA (**52**), in his studies on vegetable crops, found that the hardening process was accompanied by a marked increase in the water-retaining power of the tissues, due primarily to an increase in the imbibitional forces of the cell. He found that pentosans accumulate in cabbage and a few other vegetables during the "hardening off" process, and that this accumulation may be brought about either by exposure of the roots to deficient soil water for typical growth or by exposure to low temperatures. He used the dilatometer freezing method of FOOTE and SAXON (**16**) to determine the quantity of "free" and "unfree" water in the tissue. This method is an indirect measurement of the sum of the osmotic and imbibition pressure of the plant cells.

NEWTON and BROWN (**44**) found that the percentage of proteins and pentosans in the total dry substance of wheat plants does not increase in the fall during the hardening process, and that the marked rise in the concentration of these substances in the extracted plant juice is due to a reduction in the moisture content of the tissue. These investigators found the proteins to constitute the bulk of cell colloids, pentosans being restricted almost entirely to the structural parts of the plant. It is altogether probable that pentosans of the cell wall play a rôle in drought and cold resistance. As has been previously pointed out in connection with the ability of the younger leaves to retain their water content during wilting, these water binding substances may be in different quantities in old and young cell walls. DOYLE and CLINCH (**10**) also found that there is no apparent connection between cold resistance and pentosan content of the leaves of conifers. Thus the work of these investigators indicates that pentosans play little if any part in cold and drought resistance of certain plants.

Further experiments have been carried on to determine the effect of soil moisture on the percentage of bound water in milo and cabbage. The

bound-water method for the cabbage plant has been checked against the "hardening off" process by low temperatures. The low temperature method has been used by HARVEY (**28**) and others. Cabbage plants were used because they can be hardened easily, the evidences of freezing injury can also be readily seen, and varieties with known and relatively uniform hardening capacity are available. Milo is also known to be resistant to drought. The milo seed was secured from the University of Arizona. Four varieties of cabbage were used: Early Jersey Wakefield, Charleston Wakefield, Copenhagen Market, and Late Drumhead. Only a few varieties are adapted for early spring and fall planting. In the region of Norfolk, Virginia, the Jersey Wakefield, Copenhagen Market, and Charleston Wakefield have been found superior in winter resistance; therefore studies have been made to determine the reasons for this superiority in winter resistance and to test the possibly important factors of drought and cold resistance.

Two constant temperature chambers were used for securing the low temperatures desired. These chambers were equipped with accurate electrical temperature controls and the low temperatures established by refrigeration. A light was left burning in the chambers so that etiolation did not occur during the "hardening off" process. Great care was taken to eliminate differences in exposure of the plants to this light.

Instead of pre-freezing the tissue used (table XV) with solid carbon dioxide, the method of grinding in a food grinder was used before extraction under pressure. The results presented show in general that cabbage and milo when grown in a soil with abundance of available moisture exhibit a smaller tendency toward the development of hydrophilic colloids, thus the decreased percentage of bound water; whereas when the plants are provided with less available soil moisture or subjected to periodical wiltings the onset of drought conditions causes a greater proportion of the water in the cells to be changed from a free to a bound state. NEWTON'S (**20**) data indicate that the elaboration of the hydrophilic colloids does not take place under greenhouse conditions, but is the result of subjecting the plants to a period of progressively decreasing temperatures. ROSA'S (**52**) work shows that pentosans accumulate in cabbage as a result of depriving the roots of available moisture. The present findings substantiate those of ROSA, since the hydrophilic colloids are increased, as indicated by the increase in percentage of bound water when the plants were subjected to periodical wiltings.

The data given under "concentration" in table XV indicate considerable increase of dry substance in plants exposed to drought "hardening off" conditions. This is especially noticeable in the more hardy varieties, as Early Jersey Wakefield. Milo also shows a considerable increase in dry substance. In general the water content may be said to decrease in drought

TABLE XV

DATA ON CABBAGE AND MILO GROWN IN THE GREENHOUSE AT DIFFERENT SOIL MOISTURE CONTENTS

DATE (1931)	PLANT	AGE OF PLANT	SOIL MOISTURE	CONCENTRATION BY REFRACTION	FREEZING-PT. DEPRESSION	BOUND WATER
	Cabbage	*days*	*per cent.*	*per cent.*	Δ	*per cent.*
Mar. 3	Charleston Wakefield	45	15.9	8.7	1.087	5.22
Mar. 3	Copenhagen Market	45	15.9	8.1	0.964	5.35
Mar. 5	Late Drumhead	47	15.0*	8.6	1.022	5.13
Mar. 5	Early Jersey Wakefield	47	15.0*	8.2	0.892	6.53
Mar. 10	Early Jersey Wakefield	52	14.0	8.4	1.072	7.90
Mar. 10	Late Drumhead	52	14.0	8.9	1.139	6.80
Mar. 13	Late Drumhead	55	Periodically wilted	10.6	1.335	6.10
Mar. 13	Charleston Wakefield	55	" "	10.2	1.347	7.30
Mar. 13	Early Jersey Wakefield	55	" "	11.0	1.208	9.53
Mar. 17	Milo	40	" "	8.9	1.069	6.89
Mar. 17	Milo	40	15.0	7.8	0.690	4.30
Feb. 9	Milo	62	17.0	6.2	0.659	3.61

Coefficients of correlation	*n*	*r*
Concentration and osmotic pressure	12	0.94 ± 0.022
Concentration and bound water	12	0.66 ± 0.109
Osmotic pressure and bound water	12	0.54 ± 0.13

* An equal number of plants chosen from chambers of 14, 15, and 16 per cent. soil moisture.

and cold-hardened plants in proportion to the extent to which their growth is checked by the hardening treatment. It is obvious that any hardening process greatly influences growth. This has been noted previously in the study of periodical wilting.

The depression of the freezing point was somewhat greater for the drought-hardened plants. Similar results have been obtained by other investigators, as CHANDLER (**7**), HARVEY (**27**), ROSA (**52**), and others, most of whom worked with cold-hardened plants. The differences found here are somewhat greater than those found by HARVEY and CHANDLER. This is perhaps due to the severe treatment used.

One of the most important factors involved in the increased osmotic concentration of hardened plants is the decrease in moisture content; another is the increase of sap solutes, as sugars; and, lastly, an increased amount of water in the bound state. A number of investigators have found

an increase of soluble sugars in plants exposed to low temperatures, and recently ILJIN (**31**) reported this to be true for plants growing in very dry habitats.

It may be pointed out that both drought- and cold-hardening processes in plants are accompanied by a marked increase in the water-retaining power of the cells that enables them upon freezing to retain a large proportion of their water content in the unfrozen condition or state. Figure 5

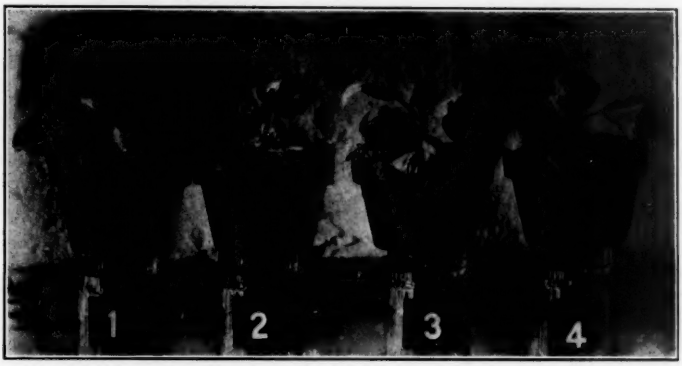

FIG. 5. Showing results of drought and cold hardening: nos. 1 and 2 are Copenhagen Market; nos. 3 and 4 are Late Drumhead. Nos. 1 and 3 hardened by exposure to severe wiltings; nos. 2 and 4 hardened by placing in cold chamber.

shows four plants of two different varieties, Late Drumhead and Copenhagen Market: (1) Copenhagen Market hardened by exposure to severe wiltings; (2) Copenhagen Market hardened by placing in a cold chamber which was maintained at 3.5° C. for 5 days; (3) Late Drumhead, given treatment identical with that of no. 1; (4) Late Drumhead, given same treatment as that of no. 2. These plants after being hardened were placed in a cold chamber, which was maintained at a temperature of −4.5° C. for 18 hours. They were then transferred back to a chamber at 3.5° C. for a few hours, before removing to the greenhouse. The plants were photographed the following day. These facts afford further evidence of a close internal connection between the phenomena of freezing and desiccation.

Hardy varieties of cabbage possess the potentiality to initiate these changes which aid in retaining the water content of the cells against desiccation, whether it be by freezing or drying. However, there seems to be a slight difference in ability, even among so-called hardy varieties. Cabbage and milo harden readily, and certain varieties of cabbage can withstand drought and quite low temperatures, but soy bean seems to lack this potentiality.

Figure 6 shows the differences in the varieties of cabbages to withstand low temperature. The plants shown in this photograph were representative ones chosen from those studied on March 13 (table XV), with the addition of the variety Copenhagen Market. This comparison of the two

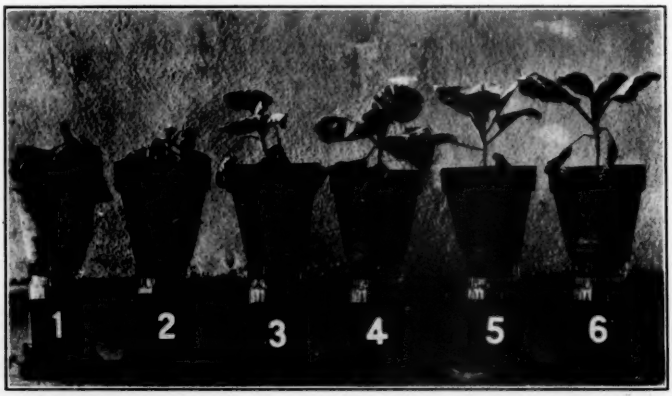

FIG. 6. Showing differences in the varieties of cabbages to withstand low temperatures: nos. 1 and 2 are Charleston Wakefield; nos. 3 and 4 are Copenhagen Market; nos. 5 and 6 are Early Jersey Wakefield.

methods for the study of hardening in plants was made to see whether any correlation existed between the percentage of bound water, as determined by juice analyses, and the ability of these varieties to withstand low temperature. The plants shown in figure 7 were placed in the chamber maintained at a temperature of $-4.5°$ C. for 12 hours. The photograph was taken the following day. Plants nos. 1 and 2 are Charleston Wakefield; nos. 3 and 4 are Copenhagen Market. Sap analyses were not made on these latter plants, but they were given identical treatment with those compared. Nos. 5 and 6 are Early Jersey Wakefield. There is a correlation between the two methods of determining the ability to retain their water contents. The Early Jersey Wakefield plants were the only ones that survived this treatment and continued their growth processes.

Figure 7 shows the effect of variation in soil moisture on cold resistance of two varieties of cabbages. Plants nos. 2, 4, and 6 are Early Jersey Wakefield; and nos. 1, 3, and 5 are Late Drumhead. Plants 1 and 2 were grown in a soil with 14 per cent. moisture; nos. 3 and 4 with 15 per cent.; and nos. 5 and 6 with 16 per cent. The plants were subjected to a temperature of $3.5°$ C. for 5 days, then transferred to a temperature of $-4.5°$ C. for 12 hours. All of the plants died in a few days, with the exception of the

Early Jersey Wakefield plants which were grown at 14 per cent. soil moisture, and these finally died after a period of two weeks. Both methods of study place the varieties of cabbages used in the order named, so far as hardiness is concerned: Early Jersey Wakefield, Copenhagen Market, Charleston Wakefield, and Late Drumhead.

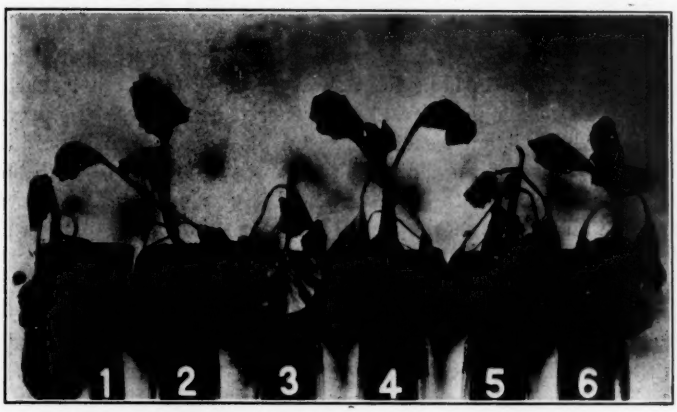

FIG. 7. Effect of variation in soil moisture on cold resistance of two varieties of cabbage: nos. 2, 4, and 6 are Early Jersey Wakefield; nos. 1, 3, and 5 are Late Drumhead.

Recently HARVEY (**29**) studied the effect of different treatments for hardening plants, using a single uniform variety of cabbage. From this investigation he suggests that hardiness in plants is a cold shock response.

VI. Correlations of various factors

FREEZING-POINT DEPRESSION AND LEAF TEMPERATURE

Values for the freezing point of the extracted sap and the leaf-temperature departures were obtained for leaves in age groups from 30 to 4 days; these data are presented in tables VI, VII, and VIII. The leaf temperatures and freezing-point values for these groups are found to be correlated to the extent of -0.61 ± 0.15. The correlation is not marked and does not indicate a very close relationship between the changes in leaf temperature and the concentration of the extracted sap. LIVINGSTON (**37**) and DRABBLE and DRABBLE (**11**) pointed out that an increase in the concentration of a solution does not reduce the vapor pressure enough to reduce effectively the rate of evaporation. On the other hand, EATON (**12**) found a high correlation between leaf temperature and depression of freezing point, and he concludes that low sap concentrations are conducive to high transpira-

tion rates and reduced leaf temperatures. It should be realized that his correlations are based on leaves studied on one date and time, namely, September 2, between 2:17 and 2:47 P. M. Data of table V show that if collections are made at different times in the day the gradient of the osmotic concentration will be greatly influenced; however, in none of the determinations on leaf temperature were older leaves found to be cooler than the younger (upper) ones.

Any mechanism which limits evaporation makes high osmotic pressure less necessary. Data in table IX show that young (upper) leaves retain their turgidity for a longer period than older (lower) ones. As previously stated, it is believed that osmotic pressure plays only a small part in this phenomenon. None of the data in this investigation seems to substantiate EATON'S conclusions on the relationship between extracted sap and leaf temperature and that osmotic pressure plays the important part in retaining the water within the leaf cells.

The coefficient of correlation r between the properties measured, and the number of cases n on which they are based, are given for the cabbages and milo at the end of table XV. The properties measured were concentration of the juice, osmotic pressure, and percentage of bound water.

The concentration of juice and osmotic pressure are found to be correlated to the extent of 0.94 ± 0.022; the concentration and percentage of bound water to the extent of 0.66 ± 0.109; and the osmotic pressure and bound water to the extent of 0.54 ± 0.13.

There seems to be no marked correlation between osmotic pressure and percentage of bound water. This indicates that while both of these factors may be related to drought and cold hardiness, they cannot both be a direct index to this phenomenon.

It was possible in most cases to classify the varieties of cabbages as to their ability to withstand low temperature by determining the percentage of bound water in the extracted juices. On the other hand, osmotic pressure afforded no marked indication of ability to withstand low temperature. Frequently the osmotic pressure would be greater for the variety which gave the lowest percentage of bound water and for the plants which were the most easily affected by the low temperature. Osmotic pressure is therefore probably not characteristic, but controlled mainly by habitat conditions. The soy beans and cabbages that were grown under nearly identical conditions showed only slight variations in osmotic pressure. It is strongly indicated that osmotic pressures are largely the result of environment.

The percentage of bound water of the fresh juice seems to be the best index of drought and cold hardiness, with concentration of the juice showing considerable relation to moisture adaptations. It is not intended to

discount the importance of osmotic pressure in the water relations of plants, but this relationship has probably been over-emphasized. There seems to be little doubt that osmotic pressure and imbibition pressure are closely connected in the water relations of plants. There are also some indications that osmotic pressure varies characteristically in certain ecological types; it seems to be slightly higher in the soy bean and cabbage than in the sunflower when grown under as nearly identical conditions as possible. It should also be pointed out that the percentage of water seems to be influenced by the percentage of soil moisture in which the plants are grown. However, the percentage of bound water seems to be more stable than the osmotic pressure.

VII. Summary

1. The more important methods which have been used in the treatment of leaves before the extraction of tissue fluids are presented. Experimental data are given in an attempt to evaluate these methods as to their advantages and disadvantages.

2. The moisture content may be read approximately by a refractometer, provided there is applied a suitable correction which has previously been determined experimentally.

3. Extracted plant juice stored at 0° and 20° C. increased gradually in osmotic pressure, as determined by the freezing-point depression, with time and the respective temperatures. The technique followed in this investigation largely avoids any errors which might be due to these causes.

4. With the approach of maturity, the moisture content of the leaf tissue decreased rather rapidly, causing corresponding increase in concentration and osmotic pressure of the sap.

5. The osmotic gradient within the plant was found to be just the reverse, when collections were made at 10:30 A. M. and 4:00 P. M. respectively. The freezing-point depression of the extracted tissue fluids decreased from the older leaves to the younger, for the material collected at 4:00 P. M.; whereas it increased from the older leaves to the younger, for the material collected at 10:30 A. M. A partial explanation for this difference is proposed.

6. The leaf-temperature measurements were made by the thermo-electrical method, in terms of the departures of the leaf temperature from the temperature of the air. The younger leaves were found to be cooler than the older ones. The leaf temperatures and the freezing-point values were found to be correlated to the extent of -0.61 ± 0.15. This correlation is not marked and does not indicate a very close relationship between the changes in leaf temperatures and the concentration of extracted sap.

7. Periodical wilting has a marked effect upon plant growth, structure, and properties of plant juice.

8. No marked differences were noted in varieties of soy beans as to osmotic concentration, ability to withstand wilting or chilling, and time of flowering, when grown under nearly uniform conditions.

9. Determinations were made of the concentration of the juice, osmotic pressure, and bound water of expressed sap of the leaves of milo and different varieties of cabbages. The percentage of bound water was determined on the juice of these cabbages, and at the same time a few representative plants were placed in a cold room. The two different methods of treatment show a high correlation.

10. On the basis of bound-water studies, elaboration of the hydrophilic colloids is thought to occur under greenhouse conditions, when the plants are subjected to periods of progressively decreasing soil moisture.

11. The varieties of cabbages could be classified very satisfactorily with respect to withstanding low temperature, on the basis of the bound-water content of the fresh juice.

12. It is believed that a high colloidal content reduces the rate of abstraction of water from the leaf tissue when exposed to low temperature or high transpiration rates, and probably confers upon these leaves the ability to withstand desiccation caused either by evaporation or ice formation.

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COMPOSITION OF CURRENT AND PREVIOUS SEASON'S BRANCH GROWTH IN RELATION TO VEGETATIVE AND REPRODUCTIVE RESPONSES IN *PYRUS MALUS* L.[1]

WALTER THOMAS

(WITH FIVE FIGURES)

I. Introduction

The purpose of this experimental project was to determine the response of apple trees to different combinations of the principal fertilizer constituents, nitrogen, phosphorus, and potassium, but with the elimination of the variables usually present in most field experiments. The phase of the investigation reported in this paper is concerned with the association of vegetative and reproductive responses to the internal conditions as represented by analysis of metabolically active tissues at different periods in the growth cycle.

1. Principal nutrient elements

Although, as is pointed out by LIVINGSTON (**96**), it is conceivable from the physiological viewpoint that conditions may be found under which any element other than the traditional ten is essential to growth, experience has shown that cultivated plants are, under ordinary field conditions when the percentage base saturation (**152**) is not the limiting factor, far more sensitive to changes in the nitrogen, phosphorus, and potassium content of soils than to any of the other elements. There is evidence (**2, 139, 165**) that the peculiar and characteristic effects of nitrogen, phosphorus, and potassium may be due to the greater motility of their ions and, in the case of potassium, to the effect on the streaming rate of protoplasm. COLLA (**30**), for example, finds that potassium has a greater effect in increasing the streaming rate of the protoplasm of *Chara crinita* than sodium or magnesium. But, whatever may be the mechanism of their action, the existence of the fertilizer industry is sufficient evidence of the agricultural economic importance of these three elements. From the standpoint of behavior and function, there is great need for more definite information concerning the rôle played by each of these dominant nutrient elements with respect to growth, reproduction, and other physiological functions.

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2. Sequence of distribution of variations in concentration of elements with respect to time

The experiences of the last decade (**18, 46, 47, 69, 85, 86, 87, 88, 89, 90, 91, 174, 201**) have confirmed and extended the value of the earlier investigations (**230, 231**) in which the concentration of certain elements or groups of elements was determined in the metabolically active tissues at critical periods in their vegetative development. Investigations of this type have proved of special service in the diagnosis of the nutritive conditions produced in plants by the application of *different combinations* of the principal nutrient elements. In this way we may describe metabolic processes with reference to their sequence of changes with respect to time. Although this method of approach does not necessarily enable the "causal relations" (**97**) to be determined but probably only the "residual effects" (antecedent influential conditions), the knowledge obtained from studies of this type supplies a valuable picture of the dynamics of physiological processes. Experiments planned in this manner have already enabled the investigator to detect quantitatively the lack of optimum (well-balanced) nutritive conditions and to prescribe remedial measures (**208**).

The sequence of changes with respect to the chemically reactive ions or reactive groups determined in this manner is not sensibly influenced by the range of variation in the meteorological conditions hitherto encountered in experiments with fruit trees. This is indicated by the similarity in direction of and relative magnitude of the seasonal changes of reactive groups or complexes, such as glucose, sucrose, starch, and also of the elements potassium and phosphorus in the present experiments compared with those reported under widely different meteorological conditions for the same species by others (**69, 122**). The writer has also obtained indications (**201, 202**) that the seasonal variations (*i.e.*, the time-concentration-curve) of the various nitrogen fractions throughout a vegetative cycle have also been affected primarily by internal causes.

3. Mechanism of fertilizer action

The present investigation is characterized by a remarkably rapid but differential response of the plants to relatively small applications—low concentrations—(page 396) of the elements nitrogen, phosphorus, and potassium, the response varying according to the combination in which these elements are present. Marked differentiation between the trees of certain treatments was apparent even within a period of six weeks after application of the nutritive elements, and the differences in response became more intensified as the experiment progressed. How do such small quantities have such marked results? How have these trees grown so differently? Is the mechanism of fertilization one of compensation or of stimulation?

According to LIEBIG'S (**94**) "mineral theory," fertilizers are effective only in so far as they supply to the soil the mineral elements in which the soil is relatively deficient. In 1903 WHITNEY and CAMERON (**224, 225**) stirred the scientific world by the announcement that plants obtain their nutrients from the substances *already existing in the soil solution.* The theory was advanced that the soil solution was approximately constant in composition, *viz.,* a saturated solution of the minerals common to all soils, and that accordingly the soil solution was in *simple equilibrium* with the soil-forming minerals. The addition of fertilizers, therefore, according to these investigators, could not increase the concentration of the soil solution. Any response to applications of nitrogen, phosphorus, or potassium by plants was attributed by WHITNEY and CAMERON to their action on the physical properties of the soil, to their effect in neutralizing toxins, and also to their catalytic or stimulatory action. This view was vigorously disputed, especially by HOPKINS (**73**), and also by the Rothamsted group (**56, 168**) who showed the fallacies of this conception. Experimental evidence has now accumulated showing that the concentration and composition of the soil solution are continuously changing (**20, 66, 177, 186, 217**), being modified by several factors: (a) the amount of soil moisture, (b) the rate of absorption and utilization of elements by the plant, (c) the respiratory coefficient of the roots, and (d) the activity of the microorganisms of the soil and of the rhizosphere.

The theory of *activation* as opposed to *compensation* has recently been revived by Italian investigators, especially by MAGRINI (**108**). The latter concludes that fertilizers act to stimulate the plant to absorb certain other nutrients found in the soil. But as the sole evidence submitted in support of the theory is the observation that only a negligible quantity of the nitrogen of the subsoil is removed by the crop after each cultivation, this would seem to be insufficient to justify the conclusion.

4. EFFECTS OF SALTS ON THE SOIL SOLUTION

Numerous investigators (**43, 51, 182, 213, 218**) have shown that fertilizer salts produce highly complicated effects. They may cause marked changes in the composition of the soil solution. For example, it has been shown by the writer (**197**) that the addition of $CaH_4(PO_4)_2$ to the Hagerstown silty clay loam soil used in these experiments increased the Fe and Al in the soil solution, and that $NaNO_3$ and K_2SO_4 increased the amount of PO_4 in solution, whereas both $CaH_4(PO_4)_2$ and K_2SO_4 increased the amount of Na in solution. The effect of fertilizers, accordingly, will necessarily depend on the resultant effect on the dominant nutritive ions of the soil solution (**208**). The reciprocal effects of nitrogen, phosphorus, and potassium in relation to their absorption by plants has been treated in detail by the writer in another paper (**208**).

5. Do roots derive their nutrients from a solution much more concentrated than that obtained by displacement methods?

This question is of paramount importance, for upon its answer depends the validity of the interpretation of the results of experiments which have for their purpose the determination of the critical concentrations of essential ions, *i.e.*, the concentration with respect to any element or ion below which the total absorption by the plant per unit of time is inadequate.

Many plant physiologists suggest that the chemotropic property possessed by the fine roots causes the root to grow from regions of low concentration of solutes to regions of higher concentrations. These regions of high concentrations would, presumably, be produced initially by the action of the CO_2 and other acids formed in the metabolic processes of the microorganisms growing around the colloidal and mineral complex of the soil particles (**103**). The mechanism involved may be explained in part at least by the Gibbs-Donnan law (**34, 35, 208**) and in part by the processes discussed in detail by Mattson (**116, 117, 118, 119, 120**). Diffusion processes then would result in the formation of a series of concentric zones of progressively decreasing concentration of solutes. As the plant grows the carbon dioxide evolved in the respiration of the roots becomes the dominating factor in the dissolution of the soil particles (**207**), furthering the formation of these concentric zones of decreasing concentration. It follows that one of the fundamental problems in studies on the *interrelationship between plant and soil* is the question whether plants can or cannot absorb colloids (**204**), and whether the actual soil solution can be obtained by any of the numerous procedures proposed to achieve this purpose, such as water extraction (**17, 19, 68, 217**), pressure (**95, 125, 162, 216**), displacement (**31, 74, 148, 171, 216**), centrifugal (**15, 16**), and freezing-point (**64**) methods.

An examination of the data by Burd (**17, 19**), Hoagland (**64, 67, 68**), Millar (**121**), Parker (**148, 149, 153**), Stewart (**186**), Teakle (**195**), and also by the writer (**197**) indicates that the soil solution obtained by displacement methods gives in the case of slightly acid or neutral soils a rather close index of the magnitude of the critical concentrations for different plants of the principal nutrient ions and of the supplying power of the soil with respect to these ions. It has frequently been observed (**19, 52, 149**), however, that crop growth is not related to the concentration of phosphate in the soil solution. For a critical analysis of the anomalous position of phosphorus the reader is referred to papers by Burd and Martin (**21**), Greenhill (**52**), and Tidmore (**209**).

It is of interest to note that the critical concentration for PO_4 may be as low as 0.20 p.p.m. in flowing cultures for corn (**149, 209**), and the critical

concentration for K in the case of alfalfa and clover may be under 0.5 p.p.m., although relatively higher values for some plants are reported, *e.g.*, 1 p.p.m. PO_4 for barley (**67**) and over 4 p.p.m. K for tomatoes (**78**). It must be noted, however, that TRELEASE and LIVINGSTON (**212**) have cautioned against the attempt to determine the comparative physiological values of different nutrient solutions without a knowledge of the climatic conditions under which the tests are made.

Specific soils, of course, vary in their ability to renew a particular nutrient in the soil solution at suitable levels of concentration, *i.e.*, above the critical concentration; and this power of renewal will depend upon the resistance to decomposition of the mineral particles and to the nature of the colloid complexes. It is necessary, too, to distinguish between the two forms of soil solution: the micellar solution, being an integral part of the micelle itself, cannot be renewed.

6. ABSORPTION OF MINERAL ELEMENTS

In earlier papers the writer (**204, 205, 206, 207, 208**) has discussed some of the factors determining the absorption of nutrient elements by the plant. The effect or action of a specific ion under given conditions of light and temperature as related to its absorption and utilization within the plant is dependent upon the concentration of the media, upon the nature of the other ions present, and upon the ratio of the concentrations of equally charged ions to one another.

There are a number of experiments (**65, 109, 163, 196**) which indicate that variations in the *total concentrations* of ions may have less effect on metabolism than changes in the *proportion* of the ions. Since continual fluctuations in the concentration and ratio of the nutritive ions in the soil solution occur, it might be expected that by the processes of adaptation plants would be gradually adjusted to somewhat wide variations in this respect; or, as HOAGLAND (**67**) expresses it, "there is no doubt that all kinds of interionic effects occur during absorption, varying with the composition and concentration of the culture solution, stage of growth and climate."

The soil conditions involved in an adequate understanding of the problems connected with the interrelationship of the plant and soil have been briefly sketched here in order that the reader may keep in mind the significance of the substrate in relation to the facts brought out in this paper. Only the analyses of the plant are reported; the soil conditions have been discussed in previous papers (**198, 208**).

II. Materials and methods

1. SOIL USED

During the period 1918–20, the late WILLIAM FREAR and the writer made several attempts to obtain information regarding the effect of the

principal fertilizer elements on the internal conditions of apple trees growing in the experimental orchards then conducted by the Pennsylvania State College throughout the State. But, inasmuch as no consistent results could be obtained, this plan was abandoned for one in which soil and also stock variations were eliminated. This was accomplished by the use of boiler-plate cylinders, 42 in number, 5 feet in diameter and 5.5 feet deep, which were sunk into the ground and filled with a virgin soil (a silty clay loam of the Hagerstown series) adjoining a strip of woodland adjacent to the college experimental orchard. The complete chemical and mineralogical analyses of this soil have been reported elsewhere (**198**). Six inches of crushed limestone were placed at the bottom of each cylinder to prevent capillary rise of the surrounding soil into which the cylinders were sunk. The soil used in these cylinders was removed by layers and each horizon was thoroughly mixed during a period of many months before introduction into the cylinders in October, 1921. The total weight of soil used was 210 tons. This soil was then allowed to remain fallow for one year to permit the effects of the climatic factors on the physical, chemical, and microbiological conditions to become uniform in all cylinders.

2. Planting

In the spring of 1922, standard stocks vegetatively propagated from a single parent secured from the East Malling Agricultural Research Station, England, whip-grafted to Stayman Winesap scions, were planted. The scions were obtained from a tree whose performance was known for two bud generations (**3**). Growth measurements made at the end of the years 1922, 1923, and 1924 before fertilizer applications were made showed no significant differences between the trees in adjacent rows (**3**) (table II).

3. Fertilizer treatment

Nitrogen, phosphorus, and potassium were supplied in the form of C.P. salts, singly and also in the various combinations shown in the diagram (fig. 1). Analysis gave $NaNO_3$, 16.45 per cent. N; $CaH_4(PO_4)_2 \cdot 2H_2O$, 56.30 per cent. P_2O_5; and K_2SO_4, 53.40 per cent. K_2O. The following amounts per tree were added: $NaNO_3$, 918 gm.; K_2SO_4, 293 gm.; and $CaH_4(PO_4)_2 \cdot 2H_2O$, 534 gm., equivalent per acre of 100 trees to 35 lb. N, 35 lb. K_2O, and 70 lb. P_2O_5. If calculated in terms of percentage amounts present in the first 8 inches of surface soil (horizon A), the amounts added are equivalent to 0.0054 per cent. N, 0.0054 per cent. K_2O, and 0.0108 per cent. P_2O_5, respectively. These quantities of salts were not applied at one time; the first application was made in April, 1925, and the second on May 3, 1925.

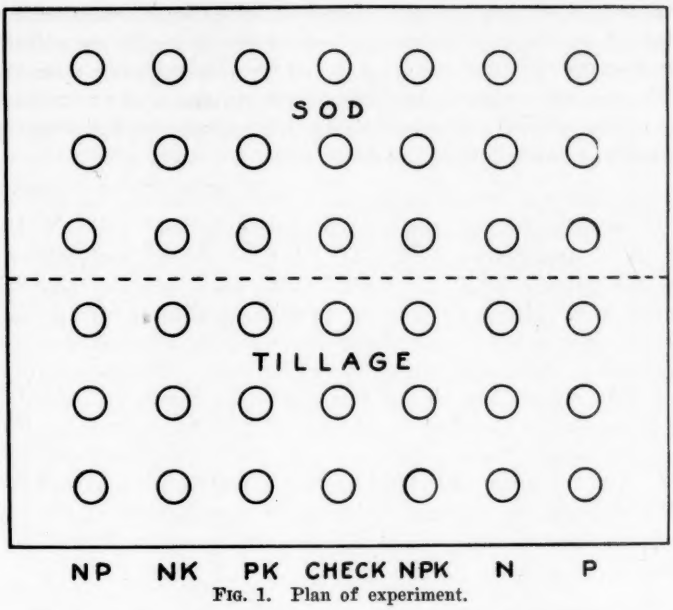

FIG. 1. Plan of experiment.

It should be noted that none of the trees bloomed before the treatment with fertilizers, *i.e.*, there were no blossoms in the spring of 1925 and, therefore, no fruit buds were formed in 1924. On May 12, 1926, the year following the fertilizer additions, the trees were deflorated, *i.e.*, the flower buds were removed just before the normal opening of flowers, to eliminate the influence of reproductive activities and fruit dominance on the growth and composition of the actively growing tissues (**9, 127, 128**).

4. SELECTION OF SAMPLES FOR ANALYSIS

Inasmuch as this investigation was not concerned with the dynamics of the photosynthetic processes, but rather with the relationship of the internal changes produced by the principal nutrient elements to the vegetative and reproductive responses, it was decided in the first instance to examine the organs used for transport of food and for storage. The question then was, to what extent does the composition of the current and previous season's branch growth reflect the changes in metabolism which resulted in such striking differences in the growth of the trees in this experiment?

KRAYBILL, POTTER, and WENTWORTH (**84**), LOOMIS (**100**), and also POTTER and PHILLIPS (**156, 157**) suggest that the samples for analytical examination should be confined preferably to new spur growth, the spurs

being nothing more than lateral branches which for some reason have made only limited growth. In the present experiment it was at the outset considered doubtful whether the spurs would provide sufficient material for the work planned. But even so there are sound reasons why investigations bearing on the internal factors associated with growth and fruit-bud formation should not be confined to the spurs alone.

A. Reasons for chemical examination of branch growth.—The chemical examination of the current and previous season's branch growth is of importance (**201**), for changes in the metabolic direction of the living cells of the woody stems must precede those in the developing bud. Hence, in the last analysis, conditions further back in the tree would *a priori* be expected to have considerable influence on the chemical system determining growth and fruiting. The investigations of Hooker (**70**) and of Hooker and Bradford (**72**), moreover, indicate that the variations in the chemical composition of bark taken from the scaffold limbs parallel closely those in the spurs, new growth, and last year's branch growth. And the writer (**201, 202**) has shown that, in so far as the nitrogen cycle is concerned, the seasonal variations in concentration of the various nitrogen fractions parallel one another in the current and previous season's branch growth.

B. Do individual branches and spurs act as a unit?—The principal difficulty in sampling fruit trees lies in obtaining homogeneous material without sensibly changing the nutritive conditions. If it could be shown that each spur acts as a unit, then errors arising from variability would cease to be much of a factor, even when only a few trees are available for analysis. Experimental and observational work exists that might seem to support the theory that individual branches and spurs act as a unit (**8, 105, 166, 226**). Thus, Auchter concluded from experiments on apple trees that mineral nutrients absorbed by the roots on one side of a plant are in large measure translocated to and used only by the trunk, limbs, and leaves directly above them; *i.e.*, there is no cross-over from one sector to another. But, as $NaNO_3$ was the only salt used in Auchter's experiments, and in view of the writer's results that nitrates are for the most part reduced in the fine roots of *Pyrus malus* (**203**), the possibility exists that Auchter's conclusions are valid only for *organic* materials (which move only in the phloem tissue) and not for the *mineral* (*i.e.*, ash) materials (**29, 111, 112, 113, 114, 115**). Neither the observations of Hardy (**57**) nor of Mack (**107**) support the view of the individuality of the spur. The latter found a uniformity of behavior of all spurs with respect to "off-year" bearing, "on-year" bearing, and "biennial" bearing.

These conflicting views may, perhaps, be reconciled when consideration is given to the fact that a fruit tree, although in effect a single organism, may be treated as a colony of competing units (**11, 62**). But even so the idea of independence can be carried too far, for a spur deprived of its own leaves can receive enough nutrient supply from adjacent parts of the tree to function actively (**62**). Moreover, the behavior of different varieties of fruit trees differs in this respect (**62**).

5. Collection of samples

On September 1, 1925, and succeeding dates (tables V to IX), samples of the current and previous season's branch growth were taken in the manner already described (**200, 201**) from the trees growing in the rims that were seeded to bluegrass on May 27, 1924, *i.e.*, from the trees growing in sod. In the following year (1926) samples were taken on April 4, June 21, August 15, and November 1. These periods may be characterized as follows: September 1, period of cessation of active growth; April 4, period of bud swelling; June 21–August 15, period of active growth; November 1, leaf fall and beginning of period of winter rest. As a matter of record, a summary of the meterological conditions is appended in table I.

TABLE I

Summary of meteorological data

Dates	Mean maximum	Mean minimum	Rainfall	Sunshine	Humidity
	° *F.*	° *F.*	*inches*	*per cent.*	*per cent.*
1925					
Aug. 15–Nov. 1	67.4	48.5	5.78	.....	81.5
1926					
Apr. 4–June 30	69.2	43.4	7.34	70.4	72.5
June 30–Aug. 15	83.0	61.4	5.35	61.4	80.3
Aug. 15–Nov. 1	68.5	50.4	12.41	.....	87.1

6. Separation of bark and wood

The bark (periderm, cortex, phloem) was separated from the wood (outer xylem, inner xylem) and each analyzed separately. In the present work no attempt was made to divide the complex constituents (tissues) of the bark and xylem, although it is realized that very limited regions may be involved in a particular response (**211, 215**).

Gradients.—There undoubtedly do exist slight differences in composition between the terminal, middle, and basal portions of the bark and wood, respectively, of shoots (**215**). But there is no evidence as yet of either a positive or negative gradient. Tufts (**215**) reported that differences in

composition between the various portions so far as location up and down the shoot is concerned are relatively insignificant, compared with those existing between bark and wood in the same portions. Preliminary work to test this point carried out by the writer prior to the present studies showed very similar results.

In the usual macroscopic method of analysis, cells of admittedly widely different nature and function are taken: epidermal, vascular, and parenchymatous tissues are mixed indiscriminately. It is, therefore, only the predominant internal relations that are revealed by the methods adopted in the present study.

7. Analytical methods

A. Do the values in total carbon determinations represent potentially mobile and metabolically reactive compounds?—In the present investigation, fractionation of the nitrogen compounds similar to those previously carried out by the writer (**201, 202**) has not been undertaken. The possibilities and limitations of nitrogen partition investigations in this species have already been clearly defined (**202**). These earlier studies have shown, moreover, that the respective nitrogen fractions were measurable functions of the total nitrogen, even under widely differing external environments. Thus, the total water-soluble N, the non-protein N, and the amino N closely paralleled the total N throughout the growth cycle; the amide N and "rest" N varied inversely with them. It would seem, therefore, that amino N must be connected with protein synthesis and "rest" N with protein degradation. Accordingly, since the sequences of variations (changes) in the distribution of partially synthesized proteins parallel the total N, the seasonal fluctuations of the former may be predicted from the time-concentration curves of the latter.

On the other hand, if any definite relationships exist between the total carbohydrate and its various fractions, they have not been identified in any metabolism studies thus far carried out. It was considered desirable, therefore, to undertake the determination of such carbohydrate fractions as are amenable to present methods of analysis rather than the determination of total carbon preferred by some investigators (**63**), who consider that the determination of total carbon is sufficient to establish the relationship existing between the carbon and nitrogen compounds present. Hicks (**63**) justifies the determination of total carbon, without fractionation according to the usual methods, on the basis that the primary value of the C/N relation lies in the fact that the growth of a plant is dependent upon the balance between the metabolic processes of carbon assimilation and respiration on the one hand and absorption of nitrogen from the soil on the other; and that, since both processes involve the use of "raw" inorganic material in the

form of carbon dioxide and nitrates, the relation of real importance in the plant itself, according to her view, is that of elemental carbon to elemental nitrogen. Although this investigator recognizes the fact that part of the assimilated carbon is transformed into immobile compounds, such as lignin, she maintains that these immobile compounds have not been shown to lie outside the vital system, and, therefore, to be without influence on the plant. It would appear that the problem of the plant physiologist is not so simple in this respect as that of the animal physiologist (**81**). Nevertheless, *the burden of proof remains with the proponents of the "total" carbon idea to show that the carbon compounds of plants are as reactive and mobile as the nitrogen compounds with which they are compared.*

B. WATER.—(1) The imbibitional water was determined by drying the material in a drying oven at a temperature of 70°–80°. The samples were afterwards left exposed to the air for several weeks and weighed when equilibrium had been reached. (2) The hygroscopic water was determined by heating 2 grams of the finely ground materials *in vacuo* at 100°.

C. ASH.—Ten grams of the finely ground material were ignited in an electric muffle furnace at a temperature not exceeding 450° to avoid loss of potassium salts.

D. POTASSIUM.—The usual LINDO-GLADDEN method was employed (**7**).

E. PHOSPHORUS.—The phosphorus was precipitated as the ammonium phospho-molybdate after the method of STEWART (**186**) and the precipitate was titrated immediately according to RICHARDS and GODDEN's modification (**164**) of the PEMBERTON-NEUMANN method (**133, 151**).

F. NITROGEN.—Nitrogen was determined by the usual KJELDAHL method (**7**). Only traces of nitrates are present in apple tissues (**38, 203**).

G. CARBOHYDRATES.—The estimation of the initial separation of carbohydrates was effected by the method proposed by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists into a "soluble" and an "insoluble" fraction (**5**). In the "soluble" portion: (1) reducing sugars (glucose, fructose, pentose, arabinose, and xylose) and (2) non-reducing sugars (sucrose and other disaccharides) were determined. Glucosides such as phloridzin (**61**) were not separately estimated; these compounds would be included in the latter group.

It is of interest to note that it has recently been questioned if phloridzin is a true glucoside. MOELWYN-HUGHES (**123**) finds that phloridzin is not hydrolyzed by emulsin but by another enzyme occurring freely in nature—probably β-(γ)-fructosidase. Moreover, he also points out that all the published data on the velocity of hydrolysis of glucosides by acids indicate that phloridzin resembles, with respect to velocity coefficient and critical increment for hydrolysis, the γ-fructosides (sucrose, raffinose, melezitose) more closely than the normal glucosides (salicin, arbutin, maltose, etc.).

The determinations of the various fractions were made according to the writer's method (**199**). The acid-hydrolyzable "insoluble" fractions (**4, 5**) include the ill-defined groups designated by SCHULZE (**172**) as "hemicellulose," and which may function as reserve material (**129, 167**). In the present investigation the term includes hexosans, pentosans, and such mixed polymers as pectins and gums.

III. Presentation of results

1. GROWTH MEASUREMENTS

Throughout the period 1922–24 inclusive, *i.e.*, before the nutrient salts were added, there was no significant difference in growth measurements in trees from different rows (**3**) (table III). The response of the trees to nitrogen additions was especially striking. Within six weeks after the first application in the spring of 1925, the leaves of the trees receiving nitrogen additions changed from a pale green to a deep green color. At the end of

TABLE II

EFFECT OF TREATMENTS ON GROWTH OF GRASS CUT IN 1925

TREATMENT	WEIGHT OF GRASS CLIPPINGS
	gm.
Check	845
NPK	5708
NP	5365
NK	2673
PK	1760
N	3743
P	1457

TABLE III

BRANCH ELONGATION IN CENTIMETERS OF SEASON'S BRANCH GROWTH (1922–1926)

TREATMENT (AFTER 1924)	SOD				
	1922	1923	1924	1925*	1926
Check	851	3270	5005	3051	985
NPK	863	2560	4662	6991	6578
NP	429	2637	4983	6475	7795
NK	648	3402	5094	5808	5031
PK	762	2517	4881	3247	2073
N	570	2796	3844	5564	5243
P	635	2696	4537	3247	1790

* First year of fertilizer application.

this period marked differences in growth responses were observed. The soil condition with respect to supply of nutrients at this time is indicated by the combined weight of the grass clippings (table II), taken in June, 1925, from the "sod" cylinders and also of the combined branch elongation at the end of the 1925 growing season (3) (table III) for all three trees in each treatment.

The effect of the differential fertilizer treatments given in the spring of 1925 on the fruit bud formation of that year is shown in table IV, which gives the number of blossom clusters removed from the three trees in each treatment in 1926 (3) when the trees were deflorated.

FIG. 2. Typical tree growing in sod treated with the three nutrient salts: $NaNO_3$, K_2SO_4, and $CaH_4(PO_4)_2 \cdot 2H_2O$.

TABLE IV

EFFECT OF TREATMENTS ON NUMBER OF BLOSSOM CLUSTERS IN 1926

TREATMENT	SOD	CULTIVATED
Check	0	6
NPK	328	353
NP	145	334
NK	102	17
PK	0	28
N	21	71
P	0	13

It may be pointed out here that the tips of the shoots of the check trees and also of those receiving only phosphorus had relatively little active cambium compared with the trees of other treatments. The nitrogen-treated trees were differentiated from the others in having less xylem with thinner walls than the trees without nitrogen additions. The cambium, too, of the trees receiving added nitrogen was active even to the base of the stem.

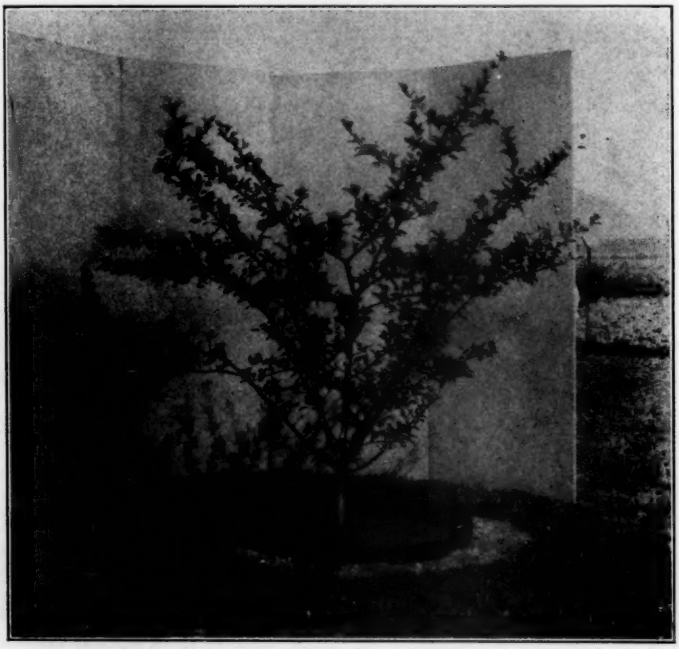

FIG. 3. Typical tree growing in sod without nutrient salt additions.

Figure 2 shows a representative tree from the row in sod and receiving all three of the nutritive elements nitrogen, phosphorus, and potassium. Figure 3 shows a representative check tree receiving no nutrient additions.

2. Analytical data

In the following tables the term "glucose" will be used to designate the reducing sugars estimated as glucose (p. 401), and the term "sucrose" to indicate the non-reducing sugars and glucosides (p. 401). The analytical results are presented in tables V–IX.

TABLE V

Percentages of water in season's and in one-year branch growth (wood and bark separated)

Laboratory no.		Sampling date	Treatment	Imbibitional water		Hygroscopic water		Total water	
Wood	Bark			Wood	Bark	Wood	Bark	Wood	Bark
7	8	Sept. 1, 1925	Check	39.42	47.00	2.23	2.72	41.65	49.72
58	59	Apr. 4, 1926		42.08	51.60	2.06	2.80	44.14	54.40
89A	89B	June 30, "		40.70	50.16	1.80	2.55	42.50	52.71
98	99	Aug. 15, "		38.80	46.70	1.40	2.70	40.20	49.40
185	186	Nov. 1, "		37.24	45.05	1.46	2.30	38.70	47.35
9	10	Sept. 1, 1925	NPK	43.11	53.70	2.54	4.05	45.65	57.75
50	51	Apr. 4, 1926		45.68	55.60	2.72	3.96	48.40	59.56
90A	90B	June 30, "		45.93	54.60	2.32	4.20	48.25	58.80
94	95	Aug. 15, "		44.10	52.90	2.40	4.80	46.58	57.70
182	183	Nov. 1, "		40.60	48.70	2.80	3.85	43.40	52.55
1	2	Sept. 1, 1925	NP	43.97	50.60	2.75	3.85	46.72	54.45
82	83	Apr. 4, 1926		44.25	55.00	2.72	3.57	46.97	58.57
85A	85B	June 30, "		45.15	52.30	2.35	3.90	47.50	56.20
110	111	Aug. 15, "		44.43	50.10	2.10	3.80	46.53	53.90
194	195	Nov. 1, "		38.70	47.60	2.02	4.00	40.72	51.60
3	4	Sept. 1, 1925	NK	42.50	50.12	2.60	3.60	45.10	53.72
74	75	Apr. 4, 1926		44.36	54.80	2.52	3.30	46.88	58.10
86A	86B	June 30, "		44.22	51.45	2.38	3.75	46.60	55.20
106	107	Aug. 15, "		43.07	48.20	2.15	3.80	45.22	52.06
191	192	Nov. 1, "		38.04	50.50	2.06	3.90	44.10	50.30
5	6	Sept. 1, 1925	PK	43.38	50.27	2.50	3.56	45.88	53.83
66	67	Apr. 4, 1926		44.26	54.70	2.08	3.76	46.34	58.46
88A	88B	June 30, "		43.10	50.86	2.30	3.85	45.50	54.71
102	103	Aug. 15, "		41.60	49.30	2.60	4.00	44.20	53.30
188	189	Nov. 1, "		40.65	49.40	2.35	3.50	43.00	52.90
11	12	Sept. 1, 1925	N	42.10	52.20	2.40	4.00	44.50	56.20
42	43	Apr. 4, 1926		42.50	55.40	2.78	3.57	45.28	58.97
91A	91B	June 30, "		44.08	54.05	2.15	4.15	46.23	58.20
95	96	Aug. 15, "		42.20	51.73	2.30	3.90	44.50	55.63
179	180	Nov. 1, "		40.50	47.80	2.25	4.00	42.75	51.80
13	14	Sept. 1, 1925	P	38.95	47.13	2.25	2.72	41.20	49.85
34	35	Apr. 4, 1926		42.38	51.10	2.20	2.65	44.58	53.75
92A	92B	June 30, "		41.28	50.18	1.72	2.40	43.00	52.58
97	98	Aug. 15, "		38.40	47.10	2.00	2.80	40.40	49.90
176	177	Nov. 1, "		37.55	46.45	1.65	2.65	39.20	49.10

TABLE VI

AMOUNTS OF GLUCOSE, SUCROSE, TOTAL SUGARS, STARCH, HEMICELLULOSE, AND "TOTAL" AVAILABLE CARBOHYDRATES AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

Laboratory no.		Sampling date	Treatment	Percentages of fresh weight of material											
				Glucose		Sucrose		Total sugars		Starch		Hemicellulose		Total available carbohydrates	
Wood	Bark			Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark
7	8	Sept. 1, 1925	Check	0.117	0.292	0.140	0.503	0.257	0.794	5.946	5.742	18.042	10.483	24.244	17.019
58	59	Apr. 4, 1926		0.229	0.401	0.078	0.410	0.307	0.812	6.173	5.016	16.021	9.758	22.500	15.586
89A	89B	June 30, "		0.104	0.189	0.035	0.355	0.138	0.544	4.859	5.490	17.290	10.427	22.287	16.462
98	99	Aug. 15, "		0.167	0.278	0.114	0.430	0.281	0.708	5.532	6.527	18.299	11.132	24.111	18.368
185	186	Nov. 1, "		0.337	0.400	0.153	0.316	0.490	0.716	6.743	6.349	17.783	11.583	25.017	18.649
9	10	Sept. 1, 1925	NPK	0.217	1.014	0.228	0.668	0.446	1.682	4.033	3.046	13.756	7.977	18.234	12.705
50	51	Apr. 4, 1926		0.325	0.768	0.175	0.384	0.501	1.153	4.051	1.415	11.796	7.869	16.347	10.439
90A	90B	June 30, "		0.181	0.692	0.202	0.124	0.383	0.816	1.656	2.493	11.519	8.446	13.559	11.754
94	95	Aug. 15, "		0.241	0.973	0.257	0.275	0.498	1.248	2.408	3.215	13.857	8.058	16.227	12.521
182	183	Nov. 1, "		0.464	1.205	0.255	0.664	0.719	1.869	4.415	2.159	13.324	10.425	18.457	14.453
1	2	Sept. 1, 1925	NP	0.234	1.098	0.202	0.706	0.437	1.804	4.529	3.553	12.749	8.126	17.716	13.483
82	83	Apr. 4, 1926		0.286	0.766	0.159	0.423	0.445	1.189	4.667	1.595	11.858	8.526	16.969	11.310
85A	85B	June 30, "		0.210	0.701	0.184	0.197	0.394	0.898	1.890	2.602	12.731	8.961	14.700	12.461
110	111	Aug. 15, "		0.257	1.014	0.267	0.401	0.524	1.415	2.940	3.467	12.908	8.759	16.383	13.650
194	195	Nov. 1, "		0.462	1.210	0.308	0.750	0.178	1.960	5.068	2.688	12.751	10.096	18.590	14.743
3	4	Sept. 1, 1925	NK	0.220	1.110	0.192	0.671	0.412	1.781	4.914	4.128	13.516	8.502	18.842	14.412
74	75	Apr. 4, 1926		0.297	0.859	0.133	0.453	0.430	1.311	4.249	2.263	12.499	8.988	17.179	12.562
86A	86B	June 30, "		0.203	0.717	0.235	0.215	0.438	0.932	2.429	3.069	11.983	10.022	14.851	14.022
106	107	Aug. 15, "		0.301	0.791	0.247	0.431	0.548	1.222	3.424	3.452	12.489	10.810	16.461	14.526
191	192	Nov. 1, "		0.425	1.093	0.280	0.820	0.704	1.913	3.913	2.922	13.382	11.814	17.999	15.656
5	6	Sept. 1, 1925	PK	0.162	0.254	0.179	0.323	0.341	0.577	4.654	4.178	15.522	10.125	20.517	14.881
66	67	Apr. 4, 1926		0.279	0.415	0.145	0.291	0.424	0.706	4.937	3.431	15.339	9.329	18.711	13.459
88A	88B	June 30, "		0.137	0.453	0.087	0.272	0.224	0.725	4.368	4.022	13.224	10.426	17.816	15.172
102	103	Aug. 15, "		0.206	0.318	0.156	0.294	0.363	0.612	4.788	4.483	15.507	10.839	20.657	15.934
188	189	Nov. 1, "		0.371	0.471	0.199	0.471	0.570	0.942	5.529	4.823	13.999	10.367	20.098	16.132
11	12	Sept. 1, 1925	N	0.244	0.876	0.194	0.701	0.438	1.577	4.190	4.117	15.351	8.725	19.980	14.419
42	43	Apr. 4, 1926		0.317	0.698	0.148	0.492	0.465	1.190	4.514	2.975	12.870	7.898	17.849	12.063
91A	91B	June 30, "		0.161	0.640	0.231	0.418	0.393	1.058	2.581	2.926	12.754	8.819	15.728	12.803
95	96	Aug. 15, "		0.233	0.799	0.250	0.541	0.483	1.340	3.441	3.727	14.114	9.096	18.038	14.163
179	180	Nov. 1, "		0.451	0.988	0.315	0.819	0.767	1.808	4.866	4.299	14.232	9.568	19.866	15.675
13	14	Sept. 1, 1925	P	0.129	0.376	0.112	0.707	0.241	1.083	5.733	5.667	17.910	10.105	23.885	16.855
34	35	Apr. 4, 1926		0.211	0.347	0.155	0.324	0.366	0.671	5.658	5.134	15.396	9.689	21.419	15.494
92A	92B	June 30, "		0.086	0.190	0.114	0.488	0.199	0.678	4.138	4.837	16.781	10.243	21.119	15.758
97	98	Aug. 15, "		0.161	0.301	0.131	0.326	0.292	0.651	4.765	5.882	18.363	10.341	23.423	17.149
176	177	Nov. 1, "		0.334	0.356	0.134	0.255	0.468	0.611	6.749	6.067	16.708	10.638	23.925	17.316

TABLE VI—(*Continued*)

97	98	Aug. 15, "		0.161	0.301	0.131	0.326	0.292	0.651	4.765	3.882	18.303	10.341	23.425	17.149
176	177	Nov. 1, "		0.334	0.356	0.134	0.255	0.468	0.611	6.749	6.067	16.708	10.638	23.925	17.316

TABLE VI—(*Continued*)

Laboratory no.		Sampling date	Treatment	Percentages of dry weight of material											
				Glucose		Sucrose		Total sugars		Starch		Hemicellulose		Total available carbohydrates	
Wood	Bark			Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark
7	8	Sept. 1, 1925	Check	0.20	0.58	0.24	1.00	0.44	1.58	10.19	11.42	30.92	20.85	41.55	33.85
58	59	Apr. 4, 1926		0.41	0.88	0.14	0.90	0.55	1.78	11.05	11.00	28.68	21.40	40.28	34.18
89A	89B	June 30, "		0.18	0.40	0.06	0.75	0.24	1.15	8.45	11.61	30.07	22.05	38.76	34.81
98	99	Aug. 15, "		0.28	0.55	0.19	0.85	0.47	1.40	9.25	12.90	30.60	22.00	40.32	36.30
185	186	Nov. 1, "		0.55	0.76	0.25	0.60	0.80	1.36	11.00	12.06	29.01	22.00	40.81	35.42
9	10	Sept. 1, 1925	NPK	0.40	2.40	0.42	1.58	0.82	3.98	7.42	7.21	25.31	18.88	33.55	30.07
50	51	Apr. 4, 1926		0.63	1.90	0.34	0.95	0.97	2.85	7.85	3.50	22.86	19.46	31.68	25.81
90A	90B	June 30, "		0.35	1.68	0.39	0.30	0.74	1.98	3.20	6.05	22.26	20.50	26.20	28.63
94	95	Aug. 15, "		0.45	2.30	0.48	0.65	0.93	2.95	4.50	7.60	25.90	19.05	30.33	29.60
182	183	Nov. 1, "		0.82	2.54	0.45	1.40	1.27	3.94	7.80	4.55	23.54	21.97	32.61	30.46
1	2	Sept. 1, 1925	NP	0.44	2.41	0.38	1.55	0.82	3.96	8.50	7.80	23.93	17.84	33.25	29.60
82	83	Apr. 4, 1926		0.54	1.85	0.30	1.02	0.84	2.87	8.80	3.85	22.36	20.58	32.00	27.30
85A	85B	June 30, "		0.40	1.60	0.35	0.45	0.75	2.05	3.60	5.94	24.25	20.46	28.00	28.45
110	111	Aug. 15, "		0.48	2.20	0.50	0.87	0.98	3.07	5.50	7.52	24.14	19.00	30.64	29.61
194	195	Nov. 1, "		0.78	2.50	0.52	1.55	1.30	4.05	8.55	5.55	21.51	20.86	31.36	30.46
3	4	Sept. 1, 1925	NK	0.40	2.40	0.35	1.45	0.75	3.85	8.95	8.92	24.62	18.37	34.32	31.14
74	75	Apr. 4, 1926		0.56	2.05	0.25	1.08	0.81	3.13	8.00	5.40	23.53	21.45	32.34	29.98
86A	86B	June 30, "		0.38	1.60	0.44	0.48	0.82	2.08	4.55	6.85	22.44	22.37	27.81	31.30
106	107	Aug. 15, "		0.55	1.65	0.45	0.90	1.00	2.55	6.25	7.20	22.80	22.55	30.05	30.30
191	192	Nov. 1, "		0.76	2.20	0.50	1.65	1.26	3.85	7.00	5.88	23.94	23.77	32.20	31.50
5	6	Sept. 1, 1925	PK	0.30	0.55	0.33	0.70	0.63	1.25	8.60	9.05	28.68	21.93	37.91	32.23
66	67	Apr. 4, 1926		0.52	1.00	0.27	0.70	0.79	1.70	9.20	8.26	24.86	22.46	34.85	32.40
88A	88B	June 30, "		0.25	1.00	0.16	0.60	0.41	1.60	8.00	8.88	24.22	23.02	32.63	33.50
102	103	Aug. 15, "		0.37	0.68	0.28	0.63	0.65	1.31	8.58	9.60	27.79	23.21	37.02	34.12
188	189	Nov. 1, "		0.65	1.00	0.35	1.00	1.00	2.00	9.70	10.24	24.56	22.01	35.26	34.25
11	12	Sept. 1, 1925	N	0.44	2.00	0.35	1.60	0.79	3.60	7.55	9.40	27.66	19.92	36.00	32.92
42	43	Apr. 4, 1926		0.58	1.70	0.27	1.20	0.85	2.90	8.25	7.25	23.52	19.25	32.62	29.40
91A	91B	June 30, "		0.30	1.53	0.43	1.00	0.73	2.53	4.80	7.00	23.72	21.10	29.25	30.63
95	96	Aug. 15, "		0.42	1.80	0.45	1.22	0.87	3.02	6.20	8.40	25.43	20.50	32.50	31.92
179	180	Nov. 1, "		0.79	2.05	0.55	1.70	1.34	3.75	8.50	8.02	24.86	19.85	34.70	32.52
13	14	Sept. 1, 1925	P	0.22	0.75	0.19	1.41	0.41	2.16	9.75	11.30	30.46	20.15	40.62	33.61
34	35	Apr. 4, 1926		0.38	0.75	0.28	0.70	0.66	1.45	10.21	11.10	27.78	20.95	38.65	33.50
92A	92B	June 30, "		0.15	0.40	0.20	1.03	0.35	1.43	7.26	10.20	29.44	21.60	37.05	33.23
97	98	Aug. 15, "		0.27	0.60	0.22	0.65	0.49	1.30	8.00	11.74	30.81	20.64	39.30	34.23
176	177	Nov. 1, "		0.55	0.70	0.22	0.50	0.77	1.20	11.10	11.92	27.48	20.90	39.35	34.02

TABLE VII

AMOUNT OF ASH, NITROGEN, K_2O, AND P_2O_5 IN FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF FRESH WEIGHT OF MATERIAL							
				ASH		NITROGEN		K_2O		P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	0.957	3.469	0.088	0.327	0.108	0.346	0.118	0.129
58	59	Apr. 4, 1926		0.754	2.868	0.152	0.424	0.106	0.319	0.103	0.091
89A	89B	June 30, "		0.661	2.781	0.166	0.364	0.129	0.369	0.079	0.097
98	99	Aug. 15, "		0.927	3.087	0.111	0.364	0.119	0.379	0.102	0.116
185	186	Nov. 1, "		1.017	3.596	0.155	0.432	0.116	0.411	0.106	0.111
9	10	Sept. 1, 1925	NPK	0.935	3.207	0.261	0.393	0.136	0.404	0.136	0.129
50	51	Apr. 4, 1926		0.877	2.588	0.397	0.582	0.137	0.549	0.116	0.104
90A	90B	June 30, "		0.828	2.472	0.422	0.478	0.145	0.476	0.099	0.108
94	95	Aug. 15, "		0.899	2.939	0.321	0.474	0.134	0.431	0.128	0.149
182	183	Nov. 1, "		1.030	3.748	0.351	0.702	0.169	0.607	0.167	0.185
1	2	Sept. 1, 1925	NP	0.938	3.316	0.267	0.409	0.109	0.323	0.132	0.132
82	83	Apr. 4, 1926		0.875	2.631	0.379	0.539	0.125	0.348	0.117	0.104
85A	85B	June 30, "		0.840	2.694	0.409	0.517	0.123	0.398	0.092	0.110
110	111	Aug. 15, "		0.866	2.914	0.353	0.507	0.134	0.419	0.126	0.161
194	195	Nov. 1, "		1.186	3.520	0.412	0.600	0.169	0.465	0.178	0.186
3	4	Sept. 1, 1925	NK	0.961	3.332	0.253	0.213	0.118	0.421	0.109	0.123
74	75	Apr. 4, 1926		0.797	2.614	0.339	0.267	0.119	0.417	0.099	0.092
86A	86B	June 30, "		0.812	2.710	0.350	0.294	0.149	0.576	0.078	0.101
106	107	Aug. 15, "		0.887	2.968	0.264	0.231	0.131	0.489	0.108	0.127
191	192	Nov. 1, "		0.950	3.648	0.313	0.278	0.162	0.571	0.113	0.142
5	6	Sept. 1, 1925	PK	0.963	3.232	0.203	0.360	0.124	0.332	0.128	0.132
66	67	Apr. 4, 1926		0.789	2.575	0.280	0.407	0.131	0.353	0.119	0.102
88A	88B	June 30, "		0.699	2.649	0.290	0.403	0.145	0.451	0.079	0.112
102	103	Aug. 15, "		0.809	2.956	0.246	0.364	0.106	0.411	0.103	0.124
188	189	Nov. 1, "		0.912	3.249	0.286	0.419	0.145	0.436	0.117	0.134
11	12	Sept. 1, 1925	N	0.932	3.053	0.244	0.394	.....	0.311	0.126	0.125
42	43	Apr. 4, 1926		0.821	2.585	0.375	0.501	0.120	0.304	0.111	0.099
91A	91B	June 30, "		0.758	2.541	0.409	0.422	.....	0.378	0.076	0.100
95	96	Aug. 15, "		0.888	2.817	0.297	0.430	0.128	0.346	0.111	0.106
179	180	Nov. 1, "		0.967	3.441	0.313	0.511	0.146	0.395	0.119	0.125
13	14	Sept. 1, 1925	P	0.929	3.480	0.106	0.376	.....	0.341	0.126	0.130
34	35	Apr. 4, 1926		0.798	2.775	0.202	0.402	0.102	0.333	0.116	0.093
92A	92B	June 30, "		0.684	2.802	0.205	0.379	.....	0.382	0.083	0.099
97	98	Aug. 15, "		0.924	3.131	0.163	0.376	0.107	0.371	0.113	0.116
176	177	Nov. 1, "		1.046	3.507	0.182	0.407	0.106	0.412	0.131	0.109

TABLE VII—*(Continued)*

176	177	Nov. 1, "		1.040	0.350						

TABLE VII—(*Continued*)

AMOUNT OF ASH, NITROGEN, K_2O, AND P_2O_5 IN FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF DRY WEIGHT OF MATERIAL							
				ASH		NITROGEN		K_2O		P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	1.64	6.90	0.151	0.65	0.185	0.689	0.203	0.256
58	59	Apr. 4, 1926		1.35	6.29	0.273	0.93	0.190	0.700	0.185	0.200
89A	89B	June 30, "		1.15	5.88	0.288	0.77	0.225	0.780	0.138	0.205
98	99	Aug. 15, "		1.55	6.10	0.185	0.72	0.200	0.750	0.170	0.230
185	186	Nov. 1, "		1.66	6.83	0.253	0.82	0.190	0.780	0.173	0.210
9	10	Sept. 1, 1925	NPK	1.72	7.59	0.480	0.93	0.250	0.957	0.250	0.306
50	51	Apr. 4, 1926		1.70	6.40	0.770	1.44	0.265	1.020	0.225	0.258
90A	90B	June 30, "		1.60	6.00	0.815	1.16	0.280	1.360	0.191	0.262
94	95	Aug. 15, "		1.68	6.95	0.600	1.12	0.250	1.155	0.240	0.354
182	183	Nov. 1, "		1.82	7.90	0.620	1.48	0.300	1.280	0.295	0.390
1	2	Sept. 1, 1925	NP	1.76	7.28	0.502	0.90	0.205	0.710	0.248	0.290
82	83	Apr. 4, 1926		1.65	6.35	0.715	1.30	0.235	0.840	0.220	0.250
85A	85B	June 30, "		1.60	6.15	0.780	1.18	0.235	0.910	0.175	0.252
110	111	Aug. 15, "		1.62	6.32	0.660	1.10	0.250	0.910	0.235	0.350
194	195	Nov. 1, "		2.00	7.48	0.695	1.24	0.285	0.960	0.300	0.385
3	4	Sept. 1, 1925	NK	1.75	7.20	0.460	0.89	0.215	0.910	0.200	0.265
74	75	Apr. 4, 1926		1.50	6.24	0.638	1.29	0.225	0.995	0.188	0.220
86A	86B	June 30, "		1.52	6.05	0.656	1.12	0.280	1.285	0.146	0.225
106	107	Aug. 15, "		1.62	6.15	0.482	1.08	0.240	1.022	0.198	0.265
191	192	Nov. 1, "		1.70	7.34	0.560	1.22	0.290	1.150	0.203	0.285
5	6	Sept. 1, 1925	PK	1.78	7.00	0.375	0.78	0.230	0.720	0.237	0.285
66	67	Apr. 4, 1926		1.47	6.20	0.522	0.98	0.245	0.850	0.222	0.245
88A	88B	June 30, "		1.28	5.85	0.532	0.89	0.265	0.995	0.144	0.242
102	103	Aug. 15, "		1.45	6.33	0.440	0.78	0.260	0.880	0.185	0.266
188	189	Nov. 1, "		1.60	6.90	0.502	0.89	0.275	0.965	0.206	0.285
11	12	Sept. 1, 1925	N	1.68	6.97	0.440	0.90	.....	0.710	0.227	0.285
42	43	Apr. 4, 1926		1.50	6.30	0.685	1.22	0.220	0.740	0.202	0.241
91A	91B	June 30, "		1.41	6.08	0.762	1.01	.....	0.905	0.141	0.240
95	96	Aug. 15, "		1.60	6.35	0.535	0.97	0.230	0.780	0.200	0.240
179	180	Nov. 1, "		1.69	7.14	0.546	1.06	0.255	0.820	0.208	0.260
13	14	Sept. 1, 1925	P	1.58	6.94	0.180	0.75	.....	0.680	0.215	0.260
34	35	Apr. 4, 1926		1.44	6.00	0.365	0.87	0.185	0.720	0.210	0.200
92A	92B	June 30, "		1.20	5.91	0.360	0.80	.....	0.805	0.145	0.210
97	98	Aug. 15, "		1.55	6.25	0.273	0.75	0.180	0.740	0.190	0.232
176	177	Nov. 1, "		1.72	6.89	0.300	0.80	0.175	0.810	0.216	0.215

TABLE VIII

RATIOS OF CARBOHYDRATE FRACTIONS, AND ALSO OF PHOSPHORIC ACID AND POTASH TO NITROGEN, CALCULATED AS PERCENTAGES OF DRY WEIGHT OF MATERIAL

LABORATORY NO.		SAMPLING DATE	TREATMENT	GLUCOSE/N		SUCROSE/N		STARCH/N		AVAILABLE CARBOHYDRATES/N		N/P_2O_5		K_2O/N	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	1.33	0.89	1.59	1.54	67.48	17.57	275.16	52.0	0.74	2.54	1.225	1.060
58	59	Apr. 4, 1926		1.50	0.95	0.51	0.97	40.48	11.83	147.55	35.8	2.48	4.65	0.696	0.753
89A	89B	June 30, "		0.63	0.52	0.21	0.97	29.34	15.06	134.58	45.2	2.09	3.76	0.781	1.013
98	99	Aug. 15, "		1.51	0.76	1.03	1.18	50.00	17.79	217.95	48.9	1.09	3.15	1.081	1.034
185	186	Nov. 1, "		2.17	0.93	0.99	0.73	43.48	14.68	161.30	43.1	1.46	3.90	0.751	0.951
9	10	Sept. 1, 1925	NPK	0.83	2.58	0.88	1.69	15.46	7.75	69.89	31.6	0.94	3.04	0.521	1.029
50	51	Apr. 4, 1926		0.82	1.32	0.44	0.68	10.19	2.43	41.14	18.6	2.64	5.58	0.344	0.708
90A	90B	June 30, "		0.43	1.45	0.48	0.26	3.63	5.22	32.15	24.0	3.48	4.49	0.343	1.172
94	95	Aug. 15, "		0.75	2.05	0.80	0.58	7.50	6.79	52.22	26.4	1.50	3.16	0.417	1.031
182	183	Nov. 1, "		1.32	1.72	0.73	0.95	12.08	3.07	52.59	20.5	1.30	3.79	0.484	0.865
1	2	Sept. 1, 1925	NP	0.88	2.68	0.76	1.72	16.93	8.67	66.23	32.9	2.02	3.10	0.408	0.789
82	83	Apr. 4, 1926		0.76	1.42	0.42	0.78	12.31	2.96	44.76	21.0	3.25	5.00	0.329	0.569
85A	85B	June 30, "		0.51	1.36	0.45	0.38	3.85	5.63	35.90	24.1	4.46	4.14	0.301	0.784
110	111	Aug. 15, "		0.73	2.00	0.76	0.79	8.33	6.84	46.42	26.9	2.81	3.10	0.333	0.709
194	195	Nov. 1, "		1.12	2.02	0.75	1.17	12.30	4.48	45.12	24.5	2.32	3.22	0.410	0.661
3	4	Sept. 1, 1925	NK	0.87	2.70	0.76	1.63	19.46	9.91	74.61	34.5	2.30	3.40	0.576	1.011
74	75	Apr. 4, 1926		0.88	1.59	0.39	0.84	12.54	4.19	50.69	23.2	3.39	5.86	0.446	0.771
86A	86B	June 30, "		0.58	1.43	0.67	0.43	6.94	6.12	42.39	27.9	4.49	4.98	0.426	1.147
106	107	Aug. 15, "		1.14	1.53	0.93	0.83	12.96	6.67	62.34	29.9	2.43	4.08	0.496	0.946
191	192	Nov. 1, "		1.36	1.80	0.89	1.35	12.50	4.82	57.50	27.4	2.76	4.28	0.518	0.943
5	6	Sept. 1, 1925	PK	0.80	0.71	0.88	0.89	22.93	11.60	101.09	41.3	1.57	2.74	0.613	0.923
66	67	Apr. 4, 1926		0.99	1.02	0.52	0.71	17.62	8.43	66.76	32.4	2.35	4.00	0.469	0.867
88A	88B	June 30, "		0.47	1.12	0.30	0.67	15.04	9.98	61.33	37.6	3.69	3.79	0.498	1.118
102	103	Aug. 15, "		0.84	0.87	0.64	0.81	19.50	12.31	84.14	43.8	2.38	3.00	0.432	1.129
188	189	Nov. 1, "		1.29	1.12	0.69	1.12	19.32	11.51	70.23	38.4	2.44	3.12	0.508	1.039
11	12	Sept. 1, 1925	N	1.00	2.22	0.79	1.78	17.16	10.44	81.82	36.5	0.85	3.16	.....	0.794
42	43	Apr. 4, 1926		0.85	1.39	0.39	0.98	12.04	5.94	47.62	24.1	2.32	5.06	0.321	0.689
91A	91B	June 30, "		0.39	1.51	0.56	0.99	6.30	6.90	38.39	30.1	4.12	4.23	.....	0.872
95	96	Aug. 15, "		0.79	1.86	0.84	1.26	11.59	8.66	60.75	32.9	1.43	4.04	0.430	0.938
179	180	Nov. 1, "		1.45	1.93	1.01	1.60	15.57	8.39	63.55	30.6	1.43	4.08	0.467	0.906
13	14	Sept. 1, 1925	P	1.22	1.00	1.06	1.88	54.17	15.07	225.67	44.8	0.15	2.88	.....	0.907
34	35	Apr. 4, 1926		1.04	0.86	0.77	0.80	27.97	12.76	105.89	38.5	0.63	4.35	0.507	0.828
92A	92B	June 30, "		0.42	0.50	0.56	1.29	20.17	12.75	102.92	41.5	0.89	3.81	.....	0.944
97	98	Aug. 15, "		0.99	0.80	0.81	0.87	29.30	15.60	143.96	45.6	0.39	3.57	0.659	0.860
176	177	Nov. 1, "		1.83	0.88	0.73	0.63	37.00	14.87	131.17	42.5	0.42	4.00	0.583	1.013

TABLE IX

RATIOS OF CARBOHYDRATE FRACTIONS TO PHOSPHORIC ACID AND POTASH, CALCULATED AS PERCENTAGES OF DRY WEIGHT OF MATERIAL

LABORATORY NO.		SAMPLING DATE	TREATMENT	STARCH/K_2O		STARCH/P_2O_5		CARBOHYDRATE/K_2O		CARBOHYDRATE/P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	55.1	16.6	50.2	44.6	224.6	49.1	204.7	132.2
58	59	Apr. 4, 1926		58.2	15.7	59.7	55.0	212.0	48.8	217.7	170.9
89A	89B	June 30, "		37.5	14.9	61.2	56.6	172.3	44.6	280.9	169.8
98	99	Aug. 15, "		46.3	17.2	54.4	56.1	201.6	48.4	237.2	157.8
185	186	Nov. 1, "		57.9	15.5	63.6	57.4	214.8	45.4	235.9	168.7
9	10	Sept. 1, 1925	NPK	29.7	7.5	29.7	23.6	134.2	31.4	134.2	98.3
50	51	Apr. 4, 1926		29.6	3.4	34.9	13.6	119.5	25.3	140.8	100.0
90A	90B	June 30, "		11.4	4.4	16.8	23.1	93.6	20.9	137.2	108.9
94	95	Aug. 15, "		18.0	6.6	18.7	21.5	121.3	25.6	126.4	83.6
182	183	Nov. 1, "		26.0	3.6	26.4	11.7	108.7	23.8	110.5	78.1
1	2	Sept. 1, 1925	NP	41.5	10.9	34.3	30.3	162.2	41.7	134.4	102.1
82	83	Apr. 4, 1926		37.4	4.6	40.0	15.4	136.2	32.5	145.5	109.2
85A	85B	June 30, "		15.3	6.5	20.6	23.6	119.1	31.3	160.0	112.9
110	111	Aug. 15, "		22.0	8.3	23.4	21.5	122.6	32.5	130.4	84.6
194	195	Nov. 1, "		30.0	5.8	28.5	14.4	110.0	31.7	104.5	79.1
3	4	Sept. 1, 1925	NK	41.6	9.8	44.8	33.1	159.6	34.2	171.6	117.5
74	75	Apr. 4, 1926		35.6	5.4	42.6	24.5	143.7	30.1	172.0	136.3
86A	86B	June 30, "		16.3	5.3	31.2	30.4	99.3	24.4	190.5	139.1
106	107	Aug. 15, "		26.0	7.0	31.6	27.2	125.2	29.6	151.8	114.3
191	192	Nov. 1, "		24.1	5.1	34.5	20.6	111.0	27.4	158.6	110.5
5	6	Sept. 1, 1925	PK	37.4	12.6	36.3	31.8	164.8	44.8	159.9	113.1
66	67	Apr. 4, 1926		37.6	9.7	41.4	33.7	142.2	38.1	156.9	132.2
88A	88B	June 30, "		30.2	8.9	55.6	35.9	123.1	33.7	226.6	135.6
102	103	Aug. 15, "		45.2	10.9	46.4	36.1	194.8	38.8	200.1	128.3
188	189	Nov. 1, "		38.0	11.1	47.1	35.9	138.3	37.0	171.2	120.2
11	12	Sept. 1, 1925	N	.....	13.2	33.3	32.9	.....	46.3	158.6	115.5
42	43	Apr. 4, 1926		44.5	9.8	40.8	30.1	148.3	39.7	161.5	121.9
91A	91B	June 30, "		.....	7.7	34.0	29.2	.....	32.7	207.4	127.6
95	96	Aug. 15, "		26.9	10.8	31.0	35.0	141.3	40.9	162.5	133.0
179	180	Nov. 1, "		33.3	10.9	42.3	34.3	136.1	39.7	166.8	125.1
13	14	Sept. 1, 1925	P	.....	16.6	45.4	43.5	.....	49.4	188.9	129.3
34	35	Apr. 4, 1926		55.2	15.4	48.6	55.5	208.9	46.5	184.0	167.5
92A	92B	June 30, "		.....	12.7	50.1	48.6	.....	41.3	255.5	158.2
97	98	Aug. 15, "		44.4	15.9	42.1	50.6	218.3	46.3	206.8	147.5
176	177	Nov. 1, "		63.4	14.7	51.4	55.4	224.9	42.0	182.2	158.4

IV. Discussion of results

1. Glucose and sucrose

Throughout the growth cycle the concentration of glucose is very much greater in the bark than in the wood; this holds for all treatments. Both glucose and sucrose decrease rapidly during the early period of rapid growth and accumulate again as growth acceleration diminishes. But the concentration of glucose (especially in the bark) in the tissues examined of the "no-nitrogen" trees is reduced considerably below those trees which were treated with sodium nitrate.

The ratio of sucrose to glucose indicates that inhibition of invertase activity is quite marked in the trees which have not received applications of nitrogen. Table X gives these data for the bark during the period of active growth.

TABLE X

Sucrose-glucose ratio

Treatment	April 4	June 30	August 15
Check	1.00	1.90	1.54
NPK	0.50	0.18	0.28
NP	0.55	0.28	0.39
NK	0.52	0.30	0.55
PK	0.70	0.60	0.92
P	0.93	2.50	1.10

The addition of nitrogen has resulted in marked effect on the activity of amylase, with concomitant increase in starch utilization. The data for the bark are brought together in table XI.

TABLE XI

Starch-glucose ratio

Treatment	April 4	June 30	August 15
Check	12.50	29.03	23.45
NPK	1.84	3.60	3.30
NP	2.08	3.71	3.41
NK	2.63	4.28	4.36
PK	8.26	8.88	14.12
N	4.26	4.58	4.61
P	14.80	25.50	17.03

These results lend added interest to Spoehr's (**178**) view that the supply of simple sugars above that required for the normal respiratory activity is one of the factors necessary for growth, and also to Loew's (**99**)

attempt to associate blossom bud formation with a definite concentration of sugars.

2. Total sugars

In all treatments the minimum concentration of sugars in the tissues examined is reached during the first period of active growth. Inasmuch as a minimum at this period has also been observed in other experiments (**69, 122, 211**), in which the external environmental factors are very different from those of the present experiment, it would appear that this phenomenon

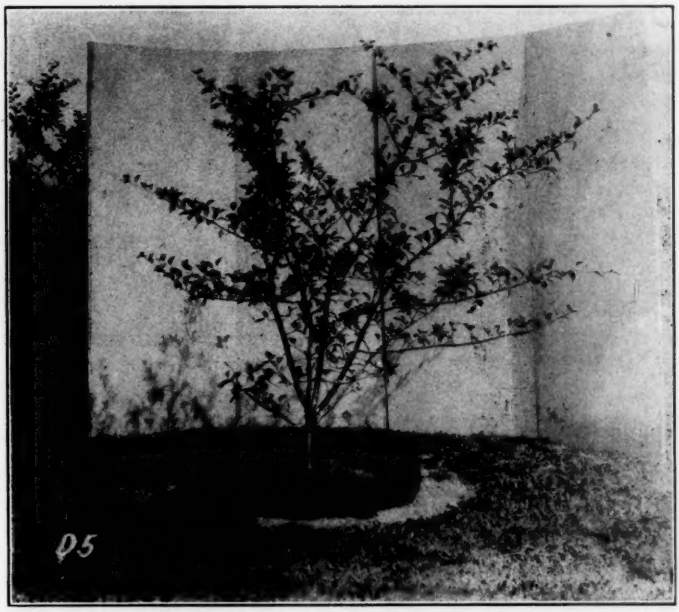

Fig. 4. Typical tree growing in sod treated with the two nutrient salts: K_2SO_4 and $CaH_4(PO_4)_2 \cdot 2H_2O$.

is independent of external factors. The concentration of total sugars in trees which did not receive added nitrogen is much lower than in the nitrogen-treated trees. The concentration of total sugars in the tissues examined as related to treatments is in the following descending order: NPK > NP > NK > N > P > check.

3. Starch

The variations in the distribution of starch throughout the cycle follow the same general course observed in other investigations with fruit trees

(**69, 122**). Comparisons in this respect with other investigations have to be made with caution, however, because only a few investigators using macroscopical methods have separated bark from wood. SWARBRICK (**194**) concludes from a review of previous work that the seasonal change in starch content shows on the whole two maxima and two minima, the maxima occurring, respectively, at leaf fall and very early in the spring; the minima at the time of maximum leaf growth and early winter. Examination of the

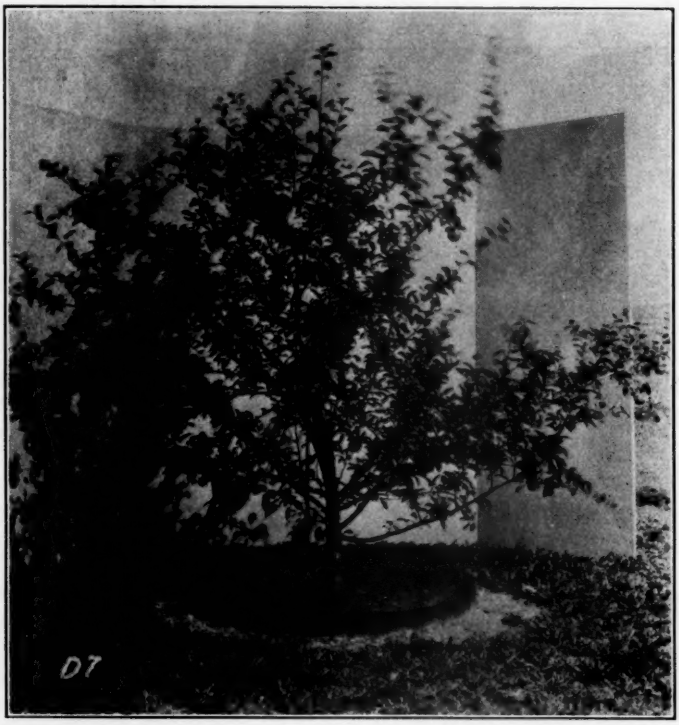

FIG. 5. Typical tree growing in sod treated with the two nutrient salts: $NaNO_3$ and $CaH_4(PO_4)_2 \cdot 2H_2O$.

results (table VI) of the present experiments shows a distinct lag of the starch changes in the wood compared with those in the bark, so that the dominant phases of the starch cycle in the wood are not the same as those for the bark. This fact has to be considered in any attempt to relate the starch cycle to other physiological phenomena, *e.g.*, to cambium activity and xylem differentiation.

The great decrease in starch which occurs in the bark—but of the nitrogen-treated trees only—is associated with swelling of the buds. This rapid utilization of starch in the spring is characteristic of the nitrogen-treated trees. Moreover, the accumulation of starch in the bark of all trees at the period of cessation of active growth and the accumulation in the wood later on in the season suggest that starch accumulation may be the cause of retardation of growth.

The starch content of the trees which received nitrogen is at a much lower level throughout the cycle than that of the trees which received no sodium nitrate applications. The trees which did not receive nitrogen show relatively small fluctuations in starch content throughout the cycle.

In this experiment there has not been observed any great deposition of starch in the cortical region of the bark found by some investigators (**161**). Microscopical examination at various times showed that the starch was distributed between the parenchymatous cells of the cortex, phloem, and medullary rays. The pith was packed with starch grains, although it was usually the last part to store it. A similar behavior has been noted by GARDNER (**44**) in the Bartlett pear. The relation of the concentration of starch to the treatments given is in the following ascending order: NPK < NP < NK < N < PK < P < check.

Energy relations of glucose and starch

Glucose and starch may be regarded as two closely related forms of potential energy (**229**). Starch is physically labile, chemically stable, non-diffusible, and non-dialyzable. Glucose, on the other hand, is chemically labile, physically stable, but diffusible and dialyzable. In the relatively low glucose concentration of the cell plasma an equally low intensity of potential energy exists, whereas starch contributes a highly concentrated form of glucose with a corresponding high intensity of potential energy. If one function of the protoplast is to act as a condensation catalyst with power of selectively removing sugars from dilute solution, this may indicate the reason why the determination of starch may be of such diagnostic value. If this view be correct, then the accumulation of starch would be the direct *cause* of growth retardation and not, as HARTWELL (**59**) suggests, the *result* of inhibited growth.

4. HEMICELLULOSES

The physiological function of this important class of structural cell wall substances is still obscure, but there is evidence to show that in many plants this group may function as a reserve material (**93, 129, 135**). Thus LEUKEL (**93**) found wide fluctuations in the amount of hemicelluloses in pasture and forage plants, and NIGHTINGALE (**135**) found a considerable decrease in

hemicelluloses in tomato plants during active growth. It must be borne in mind, however, that the sugars and starches are the more labile and, therefore, are the more indicative of immediate seasonal changes.

GERHARDT (**45**), NORMAN (**140, 141, 142**), O'DWYER (**144, 145, 146**), and SCHRYVER and his co-workers (**28**) have, in recent years, added much to our knowledge of the composition of these reserve substances. The results of these workers indicate that the hexosans (galactans, glucosans, and mannans) and pentosans (arabinans and xylans) contained in the cell walls constitute the greatest fractions in certain of the hemicelluloses. The former especially predominate in kernels of seeds poor in starch and fat. There is evidence (**45, 144, 145, 146**) that many of the hemicelluloses contain acid groups of the "uronic" and "glucuronic" acid type.

The hemicellulose of one-year old apple wood examined by GERHARDT (**45**) contained 15 per cent. lignin, 70 per cent. pentose sugars, 5 per cent. ash, and 5 per cent. moisture. No hexoses were identified. The pentosans (d-xylose and l-arabinose) were present in the molar ratio 7 : 1. However, TOTTINGHAM, ROBERTS, and LEPKOVSKY (**210**) reported that the wood of bearing spurs of apple trees contained xylose, glucose, and galactose. The preliminary hydrolysis of four products isolated from wheat bran by NORRIS and PREECE (**143**) consist of (1) a glucosan, and (2) substances containing pentose residues.

5. DO PRESENT METHODS OF HYDROLYSIS ADEQUATELY REPRESENT RESERVE MATERIAL?

The question has arisen as to whether our present methods of hydrolysis with weak or strong acids hydrolyze the reserve hemicelluloses of apple trees. BRADBURY and ROBERTS (**13**) conclude that they do not. The methods at present in use are based on the work of LECLERC DU SABLON (**92**) and of SCHELLENBERG (**169**). In apple tissues, BRADBURY and ROBERTS (**13**) found that these acids act only upon the young xylem, phloem, and cambium cells and the primary walls of other cells rather than upon the reserves occurring as cell wall thickenings. This may account for the results given in table VI, which show that the seasonal fluctuations in the hemicelluloses are relatively small and, therefore, do not afford a very sensitive index of carbohydrate changes throughout the cycle. This whole problem needs clarification.

In the present experiments hemicelluloses accumulate in the fall but no regularity or trend is observed when all the treatments are considered. The sequence of changes in the hemicellulose fraction parallels to some extent the fluctuations in starch content, but the changes in the concentrations of the latter afford, as already described, a much more sensitive index of the nutritive conditions. The reciprocal relation noted by MURNEEK (**129**) in

the reproductive organs of the apple between hemicelluloses and total sugars and also starch is not very definitely defined in the tissues examined in the present investigation. Hemicelluloses are much lower in the bark than in the wood, and are generally highest in the bark of the trees which did not receive nitrogen.

6. Total available carbohydrates

Accumulation of total available carbohydrates occurs in all trees when growth begins to slacken. The relation of the concentration of total available carbohydrates to the treatments given is in the following ascending order: NPK < NP = NK < N < PK < P < check.

The leaves were yellowish green in the trees without nitrogen additions, a characteristic of high carbohydrate and low nitrogen conditions.

7. Total nitrogen

The distribution of the nitrogen throughout the cycle is very similar to that found in the earlier experiments of the writer (**201, 202**), the characteristics of which are a high concentration of nitrogen in the spring, a decrease during the period of active growth, and accumulation in the fall. The concentration of nitrogen in the trees to which sodium nitrate was applied is much higher than in the trees which did not receive any added nitrogen. Thus, in these "no-nitrogen" trees the range is from 0.151 to 0.532 per cent. in the wood and from 0.72 to 0.98 per cent. in the bark, compared with 0.44 to 0.815 per cent. and 0.89 to 0.48 per cent. in wood and bark, respectively, of the trees which received sodium nitrate. The concentrations of nitrogen in the tissues examined from the different treatments are in the following descending series: NPK > NP > NK > N > P > check.

It has already been pointed out (p. 415) that the application of sodium nitrate has resulted in a marked increase in the utilization of starch and also (but in a smaller proportion) of the "hemicellulose" fraction. The omission of nitrogen from the complete fertilizer combination (NPK) has resulted in a *decreased* and not an *increased* absorption of the remaining elements. The significance of this has been discussed by the writer in other papers (**206, 208**).

8. Carbohydrate-nitrogen relation

The relationship of this ratio to the vegetative and reproductive responses of plants has dominated the field of horticultural research during the past decade (**6, 62, 63, 69, 71, 82, 83, 84, 135, 136, 155, 157, 167, 232**). A summary of the American investigations on this subject up to 1924 has been given by Hooker (**71**). The claims to the existence of a positive correlation between this ratio and the vegetative and reproductive responses of

plants, however, have not been allowed to pass unchallenged (**6, 232**). The whole problem is essentially related to the question of physiological balance.

A. PHYSIOLOGICAL BALANCE.—The concept of physiological balance in metabolic processes has played a considerable rôle in modern investigations in physiology (**206**). Viewing the problem with respect to the internal conditions of the plant, one may, with PEIRCE (**150**), take the position that from the observed operation of natural laws a shift in the equilibrium conditions within the living cell might be brought about *by any external factor* that influences the rate of any one of the multitude of chemical reactions taking place. Such disturbance in normal metabolism might be either temporary or permanent. We shall return to PEIRCE'S view relative to the external influence affecting fruiting later.

B. NON-EXISTENCE OF SPECIFIC CARBOHYDRATE NITROGEN VALUES FOR A GIVEN RESPONSE.—Although KLEBS (**79, 80**) made many valuable observations on the effect of alteration of external factors on blossom-bud formation, it would appear that FISCHER (**42**) was the first to suggest the possibility of using the carbohydrate/nitrogen ratio as a means of diagnosis, although no quantitative data were reported by him. His observational data led him to conclude that flowering was associated with a very high carbohydrate/nitrogen ratio whereas a low carbohydrate/nitrogen ratio favored vegetative growth.

Although there is no suggestion in the work of KRAUS and KRAYBILL (**82**) of any *specific ratio* of carbohydrate/nitrogen for any response, attempts have been made to establish limiting values and even exact mathematical ratios (**54**) between the available carbohydrate and available (total) nitrogen for a particular response. Frequent citations of GURJAR'S paper appear in the literature, but it has unfortunately been published only in abstract form. On the other hand, there is substantial experimental evidence (**62, 154**) to refute the association of a *specific* carbohydrate/nitrogen ratio with the flowering or the vegetative condition, even in the case of plants of the same species growing under different environmental conditions. Thus, a comparison of the carbohydrate/nitrogen ratios in the case of apple trees in New Hampshire (**154**) and Oregon (**62**) shows that the non-bearing spurs of the former and the most fruitful spurs of the latter investigations had the same carbohydrate/nitrogen ratio. Moreover, bearing spurs from the "sod" plot, which produced no fruit buds, in KRAYBILL'S and POTTER'S experiments (**84**) had the same carbohydrate/nitrogen ratio as the non-bearing spurs on the "nitrate" fertilized plot.

C. FOOD SUPPLY AND BLOSSOM-BUD FORMATION.—In the present investigation fruit-bud formation is definitely associated with such external factors

as the supply (concentration) of nitrogen, phosphorus, and potassium. This result is in accordance with empirical deductions of the earlier practical commercial growers and with the later more scientific field observations of GOURLEY (**50**) and of DRINKARD (**36**), that the supply of nutritive elements from the soil is *under most field conditions* the factor of greatest importance in fruitfulness. This fact is also shown by the results of the numerous experiments with laboratory control similar to those initiated by KRAUS and KRAYBILL.

The fact, however, that stunted plants of a grain field and fruit trees about to die from mechanical injury and disease may, in a season of insufficient rainfall, put forth bloom out of all proportion to the food supply, leads PEIRCE (**150**) to the view that increased absorption of mineral elements from the soil and consequently the potential supply to the *bud primordia* is not always a concomitant of fruiting. The experiments of KLEBS (**79**) leave no doubt that fruiting under the conditions of his experiments in vigorously vegetative plants was brought about by varying the supply of nutrients with changes in the illumination. In one of his experiments, for example, this was effected by lowering the concentration of salts and by increasing the illumination. But PEIRCE (**150**) would distinguish between light sufficient for photosynthesis and light as a stimulus to fruiting: "while reproduction may be accomplished without exposure to more light than sufficient for photosynthesis, the more vegetative modes will be the ones developed, the formation of the sexual organs taking place only after sufficient stimulation by light" (**150**). It is possible, nevertheless, that in the experiments of KLEBS and in the cases cited by PEIRCE, fruiting may have resulted when the proportions between available carbohydrates and nitrogen reached definite limiting values. More light would be thrown upon the interpretation of such phenomena if analytical data were available with which plants in the conditions just described could be compared with the results of experiments in which correlations of response with internal conditions are made.

D. EXTENSION OF CONCEPTION OF BALANCE TO OTHER NUTRIENT ELEMENTS.—The importance of the nitrogen supply in the maintenance of a proper relationship between the amount of vegetative and reproductive activities which has long been observed in agricultural practice, especially in fruit growing, can be accounted for by the fact that nitrogen is usually the primary limiting factor under field conditions (**71, 167**). From this viewpoint, the conception of balance may not be confined to the relations between carbohydrates and nitrogen only, but may be extended to balances between other elements or complexes, whichever may be the limiting factors to growth under the particular conditions of growth (**167, 205, 206**).

In the present investigation, and also in those reported by other investigators (**69**), carbohydrate accumulation has resulted from a deficiency of nitrogen; but accumulation of carbohydrates can result also from a deficiency of other mineral elements, especially potassium or phosphorus (**37**). That both potassium and phosphorus have exercised influence on the synthesis of carbohydrates in the present investigation is not open to question. The carbohydrate/K_2O and the carbohydrate/P_2O_5 ratios in the present investigation are shown in table IX. In relation to treatment the former follows the descending series: check = P > PK = N > NP > NK > NPK, and in the latter the order is: check > P > PK = N > NK > NP > NPK. The significance of this order of the ratios will be discussed later.

E. Experiments of Arthur, Guthrie, and Newell.—These investigators, in an extensive investigation (**6**) on the carbohydrate/nitrogen ratio as related to flowering and length of day, concluded that flowering, under the conditions of their experiments, could be initiated by a long or short day, depending on the species or variety, and that the carbohydrate/nitrogen ratio for a given species varied within wide limits *independently of flowering.* From the results obtained by them, for example, on *Lycopersicum esculentum,* these investigators concluded that the "ratio of carbohydrate to nitrogen has little or nothing to do with fruiting."

In relation to these experiments by Arthur and his colleagues there are two important points to be considered, either of which would necessarily invalidate their conclusions just mentioned:

(1) *That in order to establish the utilization of an element or compound apart from that of mere absorption, the determination of the total amounts of materials as determined by chemical methods at any one period does not differentiate between the amount available for synthesis and the amount that may have been utilized. This distinction may be determined with any degree of certainty only by means of time-absorption curves* (**208**).

(2) *That conclusions with respect to the "regulation" of the absorption of an element can be made only when the effect of varying the concentration and ratios of the other principal (dominant) nutrient elements on the absorption of the element in question is known* (**206, 208**). *In view of these facts it may be questioned whether the conclusion of these investigators that "a plant such as corn is so able to regulate the amount of nitrate taken in that it is difficult to induce this plant to take up enough nitrate to increase the total percentage of nitrogen in the plant" can be maintained.*

Arthur, Guthrie, and Newell finally suggest "that a study of enzymes or other substances present in the very small amounts in the growing tips or elsewhere offers much more promise than gross carbohydrate and nitrogen fractions in various plant organs, in explaining the mechanism of

light in initiating flowering and fruit production in the plant." Studies of this nature, especially of the type conducted by WENT (**223**), are no doubt highly desirable. It is difficult to understand, however, in what way "the study of enzymes or other substances present in very small amounts in the growing tip" will assist in determining the *causal factor* of flower bud formation. There is little doubt that much of the present confusion with regard to the relationship of carbohydrates and nitrogen to flowering and fruiting is the result of a failure, in many cases, to recognize that the ratio itself is not a *causal factor* but only the resultant of numerous antecedent unknown factors.

It is difficult to escape the conclusion from the results of the present investigation that the carbohydrate/nitrogen ratio, in particular the starch/nitrogen ratio, under ordinary field conditions serves as one of the most sensitive measurable indices of physiological balance that are at present available. In the present experiment the carbohydrate/nitrogen ratio in ascending order is NPK < NP < NK < N < P < check.

F. APPLICATION OF THE LAW OF MASS ACTION.—The concept of the reversibility of the metabolic processes of the nitrogen complexes as a function of the active mass of carbohydrates accords with the known facts of the protein-sparing action of carbohydrates (**180**). And the work of PRIANISHNIKOW (**158, 159, 160**), CHIBNALL (**23, 24, 25, 26, 27**), and also of the writer (**201, 202**) suggests the possible mechanism of this reciprocal relationship between carbohydrates and nitrogen. The final and intermediate stages of the N equilibrium may be represented: "rest" N $\rightleftarrows$ proteins $\rightleftarrows$ amino acids $\rightleftarrows$ amides $\rightleftarrows$ ammonia. The direction of the reactions would be to the right when the active mass of carbohydrates is low and to the left when the carbohydrate concentration is high (**159**).

9. ASH

The ash results present no special problem for discussion. The results consistently reflect the treatments given and are in accordance with LIEBIG'S Law of the Minimum. Thus, the series in descending order of magnitude is NPK > NP > NK > PK > N > P > check.

10. POTASSIUM

A. MECHANISM OF THE ACTION OF POTASSIUM.—Recent investigations (**75**) point to the fact that the mechanism of the action of potassium may be very complex—the resultant of photoelectric as well as of radioactive properties. In this connection it is of interest to note that LORING and DRUCE (**101**) found that the potassium chloride obtained from potatoes fertilized with KCl had an atomic weight of 40.5 $\pm$ 0.1. A qualitative test

showed this sample to be more radioactive than ordinary KCl, indicating a possible biological enrichment of K^{41} in the potato plant. This difference in the physiological effect of an isotope may be related to difference in the degree of hydration of the ions (**208**).

B. Rôle of potassium.—A review of investigations on the functions of potassium will be presented in detail by the writer in another paper. In spite of a prodigious amount of work the mechanism of the action of this element is not yet known. Why does it differ so remarkedly in its action from the chemically related element sodium? Certain physiological effects of potassium, such as its stimulating (catalytic) action on the photosynthetic mechanism, are now well established (**14, 48, 53, 60, 110, 115, 126, 137, 138, 170, 187, 192, 222, 227, 228**). Indeed, Stoklasa and his school (**187, 188, 189, 190, 191**), who have carried out the most extensive and critical experiments on the subject, maintain that synthesis of carbohydrates is possible only in the presence of the potassium ion. The presence of higher concentrations of potassium in meristematic tissues than in other tissues is regarded by some investigators as evidence of a direct or indirect function in mitosis (**104, 137**).

C. Effects of potassium deficiency.—If the results obtained with excised leaves are comparable with the living plant as a whole, as the evidence obtained by Gregory and Richards (**53**) would indicate, it would appear that the effects on assimilation of a deficiency of each of the principal nutrient elements is specific for each element. Table XII summarizes the results obtained by Gregory and Richards.

TABLE XII

Relative respiration and assimilation indices of barley in complete and deficient nutrient solutions

Treatment	Respiration	Assimilation	
		Low light intensity	High light intensity
Complete nutrient solution	Normal	Normal	Normal
Nitrogen deficient	Subnormal	Normal	Subnormal
Phosphorus deficient	Normal	Slightly supernormal	Slightly supernormal
Potash deficient	Supernormal	Subnormal	Subnormal

Thus, only the potassium-deficient leaves showed an initial fall in the assimilation rate equally marked at low as well as high light intensity. The rate of diffusion of CO_2 into the cell up to the chloroplast surface, there-

fore, would seem to depend upon the potassium concentration, possibly through its rôle in determining the bicarbonate content of the leaf.

It is of interest to compare these results with the nutrient culture experiments of MACGILLIVRAY (**106**) and of JANSSEN and BARTHOLOMEW (**77**). The former found that a deficiency of phosphorus and the latter that a deficiency of potassium resulted in increased percentage of dry matter. From this JANSSEN and BARTHOLOMEW concluded that "up to a certain concentration the potassium ion aids carbon assimilation. If, on the other hand, the concentration of potassium is increased carbon assimilation is again decreased." These anomalous results emphasize a point that plant physiologists cannot ignore; this is that experiments planned to determine the rôle of any of the nutritive elements are of little value unless attention is paid to the question of physiological balance (**208**).

If, moreover, the defense mechanism of the plant against an excess absorption of an ion such as potassium is that of precipitation, as suggested by some investigators (**104, 214**), such regulatory ability must be very limited. The work of SEKERA (**174**) is important in this connection. The clear-cut evidence from SEKERA's carefully planned experiments on barley is that normal plants are able to regulate the amount of an ion absorbed in excess of that utilized in metabolism by expulsion of that element into the soil *via* the roots. This has been discussed in detail by the writer in another paper (**208**).

D. POTASSIUM AND TRANSLOCATION OF STARCH.—Although there is considerable evidence to indicate that potassium is effective in increasing the translocation of starch (**75, 76, 77, 110, 138**), experiments may also be cited (**134, 176**) the results of which have been interpreted as indicating that translocation of starch is independent of potassium salts. Thus, NEWTON (**134**) found in water cultures that almost twice the weight of potato tubers was obtained in minus-K solutions compared with those from the complete culture solutions. The weight of tops and roots, however, was slightly in favor of the latter cultures. However, in the minus-Ca series, in spite of leaving a 25-gm. seed piece attached to the seedling, the plants died. Potato tubers have relatively a very high content of potassium and a very low content of calcium. Hence, as NEWTON points out, his experimental evidence leads to the conclusion that the beneficial results of omitting potassium were due to the increased absorption of calcium. The reciprocal action of potassium and calcium has been observed in a number of experiments (**208**). These anomalous results accordingly may be attributed to a lack of physiological balance in the potassium cultures. The results of SMITH and BUTLER (**176**) may be similarly interpreted. The experiments of the latter do not indicate that translocation of starch is independent of

potassium but that the potassium present in the seed is relatively efficiently utilized during the very short duration of these experiments (21 days).

E. Effect of potassium additions in the present experiments.—

(1) *On the leaf color.*—None of the typical symptoms of potassium deficiency, *viz.*, yellowish green leaves (the color is usually dark green if sodium is also deficient) with purple veins or browning of the periphery, were observed in any of the trees.

(2) *On the enzyme system.*—Many experiments (**33, 40, 41, 175, 184, 193**) indicate that potassium has a marked effect on the enzyme systems of the plant and that the effects are specific for different enzymes. In the present experiments potassium additions have increased the activity of amylase and of invertase (table VI). The marked effect of potassium in increasing the utilization of starch in these experiments is also apparent from the data given. Thus, the starch/K_2O ratios are in the following ascending order with respect to treatments: NPK < NP < NK < N < PK < P < check. It is of interest to note that Reed and Haas (**163**) observed that orange trees can function with less potassium than they ordinarily absorb. Trees receiving no added potassium were able to grow for 17 months subsisting presumably on the potassium in the trees at the time they were planted.

(3) *On the absorption of nitrogen and phosphorus.*—Potassium additions have increased the absorption of both nitrogen and phosphorus in the tissues examined (**205, 206**) and, as a later paper will show, this statement may be generalized to the absorption of these elements by the entire tree.

(4) *On the potassium/nitrogen ratio.*—Wallace (**219, 220**) has emphasized the significance of this ratio. In his sand cultures, leaf scorch (tip burn) of trees growing with insufficient potassium increased with each increment of nitrogen in the nutrient solution; at relatively high K/N ratios leaf scorch (tip burn) did not develop. Bronzing of the leaves of citrus trees growing in nutrient solutions having a low K/N ratio was also reported by Reed and Haas (**163**).

Certain investigators (**39, 77**) have noted a reciprocal relationship between the content of the plant with respect to nitrogen and potassium. The whole question of the mechanism of the reciprocal relationship between nitrogen, phosphorus, and potassium in relation to their absorption by plants has been discussed in detail by the writer elsewhere (**206, 208**). It may be pointed out here, however, that no generalizations are possible with respect to the K/N ratio because the absorption of these elements by the plant is a function of the level of nutrition, *i.e.*, rate of supply of nutrients (**206, 208**). Accordingly, results may show either a positive (as in the present experiment) or a negative correlation (**39, 77**) with respect to the amount of these elements absorbed by a plant.

11. Phosphorus

A. Rôle of phosphorus in respiration.—The experiments of both Lyon (**102**) and Spoehr (**179, 181**) *in vitro* indicate that phosphorus may act as an accessory accelerator of respiration. The starch/P_2O_5 and the carbohydrate/P_2O_5 ratios (table IX) of the trees of the various treatments in the present field experiments also suggest such an effect. The isolation of a reducing hexosemonophosphate by Neuberg (**131, 132**) from bottom yeast and by Barrenscheen and Pany from *Elodea canadensis* (**12**) affords very positive evidence that phosphates play a rôle in the intermediate stages of the carbohydrate metabolism of plants.

B. Absorption of phosphorus.—In the present experiments the absorption of phosphorus by trees which did not receive phosphorus application and by those trees which received phosphorus additions unaccompanied by the other principal nutrient elements is relatively low. A significant increase in the absorption of added phosphorus has occurred only by the addition of potassium or nitrogen or both, *i.e.*, in the NP and NPK treatments. Since a rapid "fixation" of $PO_4^{\equiv}$ ions takes place in this Hagerstown clay loam soil (**197**), this increase in the absorption of phosphorus may be interpreted as the result of an increase in the solubility of phosphorus following a decrease in the already existing potential difference between the micellar and intermicellar soil solution by the K^+ and NO_3^- ions, in accordance with the Gibbs-Donnan law (**1, 49, 116, 183, 185, 208**).

C. Starch/P_2O_5 ratio.—Both the starch/P_2O_5 and the available carbohydrate/P_2O_5 ratios indicate that phosphorus may act as an accessory accelerator of respiration only if adequate supplies of nitrogen and potassium are present. Hence, it follows that in soils, such as the Hagerstown clay loam used in this experiment, which are relatively low in nitrogen and phosphorus albeit high in available potassium, the application of a carrier of phosphorus (P) or of phosphorus and potassium (PK) would be ineffective to permit sufficient utilization of carbohydrates for normal growth and reproduction unless nitrogen is also applied.

D. N/P_2O_5 ratio.—In the leaves of *Vitis vinifera*, Lagatu and Maume (**87**) found a linear relationship between the nitrogen and phosphorus contents. This would be expected if all the phosphorus is in organic combination as nucleic acid, lecithin, and allied substances. The nitrogen and phosphorus would then be present in the same molecule. This linear relationship does not exist in the tissues examined in the present experiment, for wide seasonal changes in the N/P_2O_5 ratio occur in all treatments. This indicates that the large and variable proportions of the phosphorus are present as nitrogen-free compounds.

12. Water relations: physiological dryness of tissues

The position of the water balance within a plant is determined by the power of the roots to maintain the supply and the power of the leaves to resist excessive loss. The rate of water loss, if the plants do not differ internally in some manner affecting transpiration, will depend upon the nature of the leaves and the area of foliage produced. Many experiments (**22, 58, 98, 173**) indicate that the nearer to the optimum the physiological balance of the nutrient solution the smaller becomes the transpiration per unit of dry matter produced.

A. Potassium and physiological dryness of tissues.—From the observation that potassium-starved fruit trees contained less water than plants receiving potassium additions, Wallace (**221**) postulates that physiological dryness of tissues may be a characteristic of plants deficient in potassium. James (**76**) also found a significant correlation ($P < 0.01$) between potassium and water content throughout the growth period of *Solanum tuberosum* in the presence of chloride ions but not in their absence, and Gregory and Richards (**53**) found even a decreased water content associated with additional potassium at moderate concentrations. These facts would seem to indicate that other causes than osmotic pressure and solution tension are at work. Nevertheless, the influence of such a strongly hydrated element as potassium upon the swelling properties of gels must be considerable. These results are of interest with respect to the relation of potassium to chlorophyll formation and carbohydrate synthesis discussed earlier in this paper. In this connection, it is interesting to note that the work of Dastur and Buhariwalla (**32**) shows a positive correlation between the chlorophyll content and water content. Both rise as the leaf grows to maturity and both fall (the water content most) as the mature leaf grows old.

In the present experiments, nitrogen appears to be the greatest factor in decreasing physiological dryness of tissues (table V). This may be due to the influence of sodium nitrate additions on the increased uptake of potassium. The leaves and one-year and season's branch growth of trees receiving additions of all three nutrients (NPK) have the highest water content. The series in descending order is: $NPK > NP > NK = N > P >$ check.

B. Bakhuyzen's physico-chemical interpretation. — Bakhuyzen (**10**) has sought to interpret the facts of permeability, of ecological behavior, of differentiation and adaptation, and also of the life cycle of plants from the colloid chemical view-point. The water content, therefore, plays an intrinsic rôle in this theory. If the data of Mommsen (**124**) and Naylor (**130**) can be generalized, "external" protoplasm has an isoelectric point

at a pH of 4.2–4.5, whereas that of the nucleus is much higher, *viz.*, pH 5.0–5.5. There exists, therefore, a relatively high ζ-potential between "external" protoplasm and the nucleus; in other words, the peripheral plasm is more negatively charged than the perinuclear part. Accordingly, BAKHUYZEN postulates that, as the growth of the cell proceeds, the hydration capacity of the "external" protoplasm increases (increase of negative charge), whereas that of the "internal" protoplasm or perinuclear plasma decreases (decreases of negative charge or increase of positive charge). Since the minimum hydration of a colloid is at its isoelectric point, a coagulation of the internal plasma with consequent loss of hydration water and absorbed solutes occurs as growth proceeds. In this way the vacuole is formed. As the cell grows the difference between the charge (ζ-potential) of the "external" and that of the "internal" protoplasm increases up to the point when the cell begins to age. If the negative charge on the outside of the protoplasm is destroyed, solutions of acids, bases, or salts will nevertheless tend to stabilize the colloid. BAKHUYZEN argues that since OSTERHOUT (**147**) finds that death of protoplasm results in loss of isoelectric charge, there must, in long-living cells, be a force which keeps up this charge during the life of the cell. He postulates that the source of this counteracting force is probably the growth hormones, which increase the hydration capacity of the protoplasm during growth and tend to keep it up after growth in volume is over (**56, 223**). The withdrawal of water and salts from the vegetative parts (**9**) is thus accounted for.

V. Summary

Standard apple stocks vegetatively propagated from a single parent and whip-grafted to scions from a tree of known history were planted in a virgin soil contained in 5 foot by 5.5 foot boiler plate cylinders. These cylinders, 42 in number, were sunk in the ground but were isolated from the surrounding soil by means of a 6-inch layer of crushed limestone. Two years after planting, the trees were subjected to differential fertilizer treatments by the application of different combinations of the pure salts $NaNO_3$, $CaH_4(PO_4)_2 \cdot 2H_2O$, and K_2SO_4, applied in very small (low) concentrations in the spring of each year.

Marked differences in response to treatments were visible within a few months and became progressively intensified as the experiment continued. During the years 1925–1926, analyses of the one year and season's branch growth with respect to certain elements or groups of elements were made at critical periods in the seasonal growth of the trees under sod. The bark and wood were separately examined. The reproductive responses are indicated by the number of blossom clusters formed during this period. *Under cultivation:* NPK, 353; NP, 334; N, 71; PK, 28; NK, 17; P, 13; check, 6.

Under sod: NPK, 328; NP, 145; NK, 102; N, 21; PK, 0; P, 0; check, 0. Branch elongation followed the same order relative to treatment.

The analytical results on the one year and season's branch growth show that:

1. Both "glucose" and sucrose decrease rapidly during the period of active growth and accumulate again when growth acceleration diminishes; this accumulation of simple sugars appears to be a necessary precursor of growth.

2. The concentration of simple sugars in the case of trees which did not receive any sodium nitrate applications is much lower than in the nitrogen-treated trees.

3. Inhibition of invertase activity is marked in the case of the trees which did not receive nitrogen.

4. The starch cycle in the wood lags behind that of the bark. Bud swelling, especially in the nitrogen-treated trees, is associated with a great decrease of nitrogen in the bark.

5. Storage of starch up to the fall in the no-nitrogen trees is relatively very great, but the accumulated starch reserves of the trees which received applications of sodium nitrate rapidly diminish as growth proceeds. In relation to the different treatments, starch follows the ascending order NPK < NK < N < PK < P < check. Storage of starch is intimately connected with cessation of active growth and appears to be the cause of growth retardation in these experiments.

6. The energy relations of glucose and starch are examined and discussed.

7. The literature on the physiological function of the hemicelluloses as reserve material is briefly reviewed. In the present experiments the seasonal fluctuations of this group are relatively small.

8. As in the case of starch, the total available carbohydrates in relation to the different treatments follow the same ascending series: NPK < NP < NK < N < PK < P < check.

9. Total nitrogen decreases rapidly during the period of active growth and accumulates in the fall. The concentration of nitrogen in relation to the treatments follows the descending series: NPK > NP > NK > N > P > check.

10. The addition of nitrogen as sodium nitrate results in a great increase in the utilization of starch. Nitrogen concentration is the principal factor that determined, in these experiments, the utilization of accumulated starch; but increased phosphorus and potassium concentrations in the tissues examined also stimulate additional utilization resulting in increased fruit-bud formation.

11. The literature of the carbohydrate/nitrogen ratio (and also of the starch/nitrogen ratio) in relation to the vegetative and reproductive responses of plants is examined and discussed. It is pointed out that, although no mathematical ratio in the sense of the existence of a specific carbohydrate/nitrogen ratio for a particular response exists for plants growing under different meteorological conditions, nevertheless, as the present experiment indicates, this ratio serves, under the conditions ordinarily met with in the field, as one of the most sensitive measurable indices of physiological balance that are at present available. In the present experiment this ratio shows a definite gradient relative to the treatments in the following ascending series: NPK < NP < NK < PK < P < check.

12. The percentage of ash in relation to treatments is in descending order: NPK > NP > NK > PK > N > P > check.

13. The literature with respect to the rôle of potassium in plant nutrition is briefly discussed. It would appear that the mechanism of the action of potassium is very complex and is probably the resultant of photoelectric as well as radioactive properties.

14. Although the soil used in these experiments has a high potassium content (3.96 per cent. K_2O), the application of K_2SO_4 increased the potassium content of the tissues which has resulted in increased utilization of starch.

15. The reciprocal relationship between potassium and nitrogen of plants found by some investigators is discussed. A positive and not a negative correlation between these elements is found in the present experiment.

16. The application of phosphorus as $CaH_4(PO_4)_2 \cdot 2H_2O$ also resulted in increased utilization of starch. Phosphorus appeared to have assisted in the oxidation of the simple sugars.

17. The amount of phosphorus absorbed by trees which received no applications of a phosphorus carrier, and also by those trees which received phosphorus additions unaccompanied by one of the other nutrient elements, is relatively small. The reasons for this are discussed. Applications of potassium and also of nitrogen have increased the absorption of phosphorus.

18. Both the starch/P_2O_5 and the available carbohydrate/P_2O_5 ratios indicate that phosphorus may act as an accessory accelerator of respiration only if adequate supplies of nitrogen and potassium are present.

19. Although some investigators find that physiological dryness of tissue may be characteristic of potassium-deficient plants, in the present experiment nitrogen has been the greatest factor in decreasing physiological dryness of tissue. Thus, the amount of water in the tissues examined follows the descending series: NPK > NP = N > NK > PK > P > check.

20. The literature on many phases of the interrelationships between the plant and the soil pertaining to the present study is reviewed and examined.

The budding, grafting, and general care of the trees were in charge of Dr. R. D. ANTHONY and Mr. J. H. WARING, of the Department of Horticulture. The growth measurements were made by Mr. LEIF VERNER and Mr. R. H. SUDDS, also of the Department of Horticulture.

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RELATION OF AGE AND OF SEASONAL CONDITIONS TO COMPOSITION OF ROOT, PETIOLE, AND LEAF BLADE IN RHUBARB

CHARLES W. CULPEPPER AND JOSEPH S. CALDWELL

(WITH FIVE FIGURES)

Introduction

Members of the genus *Rheum* have been employed in medicine from a period antedating authentic history, and the general distribution of most of the species throughout Europe and North America is due primarily to such use. The employment of rhubarb as an article of food is relatively recent, the earliest reference to such use found by STURTEVANT (HEDRICK **17**) bearing the date 1778; but its use appears to have become rather general shortly after 1800. Excellent descriptions of the several species are given by various writers (**3, 7, 19**) in connection with directions for the cultural treatment of the crop, and there is a rather extensive literature dealing with the chemical composition of the root and the physiological action of the various constituents isolated therefrom which has recently been reviewed in connection with a comprehensive reexamination of the root (**35**). The older literature dealing with the use of the plant in medicine has been exhaustively reviewed by HEUBERGER (**18**). Such chemical studies as have been made of the aerial parts have been concerned with the determination of the food value of the petioles or with the possible toxicity of the leaf blades when employed as food. Apparently no comprehensive physiological or biochemical study of the seasonal development of the entire plant has hitherto been made.

In many respects the rhubarb plant (*Rheum hybridum* was the species studied) lends itself exceptionally well to such investigation. The large size of the several parts and the extremely rapid development of the enormous leaf blades and petioles make it suitable for studies for which large quantities of material and frequent collections are necessary. The size of the leaves facilitates marking, so that collection of material of known age is readily possible. The high acidity and low carbohydrate content of the aerial parts are exceptional, and suggest that the metabolic processes of the plant possess features of interest. In connection with studies upon the food value and cooking and canning qualities of rhubarb, some of which have been reported elsewhere (**14**), it was found that the physiology of the plant presented some unique features. It was therefore considered advisable to undertake biochemical studies for the light which the results might throw upon the problems of its utilization in cooking and in canning, as

well as for their general physiological interest. The present paper presents the results of this study.

Review of literature

The literature dealing with the chemistry of rhubarb is concerned, as already indicated, with the food value of the aerial parts or the therapeutic effects of the constituents of the root, and not with the biochemistry of growth and development of the entire plant or with the effect of climatic conditions or plane of nutrition in modifying the character or degree of these changes. The work of STEINMANN (**32**) was devoted to the determination of the titratable acidity of the petiole and leaf blade at various stages of development, to the effect of illumination and darkness upon the acid content, and to the gradient of titratable acidity between leaf mesophyll, veins, and petiole. He reached the conclusion that oxalic acid may be a product of photosynthesis, playing a rôle analogous to that of carbohydrates in ordinary green plants. RUHLAND and WETZEL (**27**) have reported studies upon the plant which they consider indicate that the high acid content is the result of deamination of proteins rather than of partial oxidation of carbohydrates.

There is an enormous literature dealing with the annual cycle of chemical exchanges between leaves and storage organs of woody perennials, and particularly of fruit trees, from which the general character and sequence of these changes can be rather completely determined. This literature has been excellently summarized by GARDNER, BRADFORD, and HOOKER (**16**), and by CHANDLER (**13**). The degree to which the results of such studies apply without modification to the rhubarb plant is questionable, since production of flower stalks and fruits is normally prevented in cultivation, storage of reserves is confined to the underground rhizome and roots, and the aerial parts are remarkable for their consistently high acidity and low content of sugars and acid-hydrolyzable polysaccharides at all stages of their development. In consequence the physiology of the plant would be expected to present some points of contrast with that of woody stemmed perennials and trees on the one hand, and with that of annuals on the other.

There is a considerable literature dealing with the chemistry of development of foliage leaves, chiefly of herbaceous perennials and trees. The results reported by OTTO and KOOPER (**24, 25**), TUCKER and TOLLENS (**34**), SWART (**33**), and LECLERC DU SABLON (**15**) are typical. SWART found that the fall of the leaf in autumn was preceded by a decrease in protein and in nitrogen, phosphorus, and potassium. He reviewed the older literature in considerable detail and presented analyses upon leaves of a great number of trees and perennial shrubs made at two stages, namely, when the leaves

were normally green and when autumnal coloration had set in. In all cases the development of coloration is accompanied by very marked decrease in total nitrogen, potassium, and phosphoric acid.

TUCKER and TOLLENS (**34**) collected leaves of *Platanus occidentalis* at intervals of 3 to 4 weeks from June 13 until November 5, and determined water and ash content and the composition of the ash. The water content was highest in the first sample and decreased gradually up to October 8, then rose slightly in the final sample. Ash increased throughout the series. The percentage of silica, calcium, magnesium, and chlorine present in the ash increased steadily through the series; phosphoric acid and potash were highest in the initial sample and fell off gradually up to September 7, then more rapidly to values less than one-half those of the initial sample. Nitrogen showed a similar but more pronounced decrease to one-fourth its original amount. TUCKER and TOLLENS reduced their results to terms of equivalent leaf area, so that the decreases are absolute and not relative. As WEHMER (**38**) had suggested the possibility that constituents of the ash were removed from the older leaves by rains, a portion of the tree was protected by a water-tight cover during rainfall from October 8 onward. The differences between protected and unprotected leaves were without significance. The reduction in potassium, phosphoric acid, and nitrogen was therefore due to transport from the leaves into the wood, not to leaching by rains.

LECLERC DU SABLON (**15**) made determinations of sugars and total carbohydrates, total nitrogen, fat, and water in root, stem, and leaf of chestnut, pear, quince, peach, and willow trees and in raspberries at intervals of 30 to 35 days throughout the year, finding that the total carbohydrate of leaves generally increased somewhat, then fell off very considerably previous to leaf fall. Total nitrogen was maximum in amount in the youngest leaves and decreased rapidly until the leaves were fully grown, then decreased more slowly. Water content was highest in the youngest leaves and decreased with age. Carbohydrates and total nitrogen of root and stem were maximum in October and November, decreased rather rapidly to a minimum in April or May, then increased from June or July to the end of the growing season.

BERTHELOT (**6**) has summarized a considerable number of analyses of various plants at several stages of development, but the distribution of nitrogen among its various forms was not determined.

The work of OTTO and KOOPER (**24, 25**), while primarily carried out with another purpose in mind, shows that there is a progressive decrease in nitrogen content of the leaves of *Syringa vulgaris, Sambucus nigra, Philadelphus coronarius,* and *Aesculus hippocastanum* throughout the season.

The literature in regard to the chemical changes in the leaves of deciduous perennials and trees is in agreement with that here cited in indicating that there is a progressive decrease in total nitrogen content of the leaf from spring to autumn, and that senescence of the leaf is preceded by a complete disappearance of nitrates and a further decrease in nitrogen, phosphorus, and potash as a result of transfer from the leaf to the stem. Concurrently with the decrease in total nitrogen there is a progressive increase in water content of the leaf, which becomes relatively stationary during the period of functional activity and declines as senescence sets in. BENEDICT (**4**), who has summarized the literature on the physiological changes occurring in senescence in leaves, found that old leaves of *Vitis vulpina* had materially lower capacity for absorbing water than young leaves, which he attributed to the increased content of vascular tissue in the old leaves.

Certain annual plants are known to accumulate very considerable quantities of nitrates in their stems and leaves. BOUTIN (**8, 9**) reported in 1873–1874 the finding of large quantities of potassium nitrate in aerial parts of *Amaranthus atropurpureus, A. blitum,* and *A. ruber.* PAMMEL and DOX (**26**) made chemical analyses and microchemical studies of *A. blitoides, A. graecizans,* and *A. retroflexus,* and the results of their total nitrogen determinations ranged between 1.88 and 2.49 per cent. As the quantities of protein indicated by the microchemical tests were in all cases very small, much the larger portion of the total nitrogen was non-protein nitrogen, and a considerable part may have been present as nitrate. These reports prompted further work by WOO (**39**) and its subsequent extension by CAMPBELL (**12**).

WOO made chemical analyses of roots, stems, branches, and leaves of *Amaranthus retroflexus* at three stages of growth, beginning at emergence from the ground and ending just before blooming. CAMPBELL (**12**) continued the work to include the mature stage and also made analyses of 25 other weed species at three stages of development. WOO had found a pronounced accumulation of nitrate nitrogen in the stems and branches with less marked increase in the roots just prior to blooming, which had entirely disappeared from all parts in the mature plant. CAMPBELL found in all the species studied that a somewhat analogous accumulation of nitrate nitrogen occurred in the early stages of growth, reaching its maximum just before blooming, then disappearing in the mature stage. Of the various orders and families represented, representatives of Chenopodiales showed the highest accumulation of nitrate, with Polygonales ranking next (represented by *Rumex crispus, Polygonum pennsylvanicum,* and *P. convolvulus*). When grown in soils very high in available nitrate content, as on piles of stable manure or decayed hog hair, *Amaranthus retroflexus* and

Atriplex patula showed a considerable content of nitrate nitrogen at full maturity. In all the forms used the total nitrogen content of stems, roots, and leaves decreased from the earliest stage to maturity.

Woo found that total carbohydrates, polysaccharides, and non-reducing sugars in roots and stems increased in the first two weeks after emerging from the soil, meanwhile decreasing in the leaves. Just before blooming the relations were reversed, a sharp decrease in these constituents in the root and stem accompanying the increase in the leaves. Total carbohydrate and total nitrogen showed reciprocal fluctuations in each of the three parts, increase in total nitrogen accompanying decrease in total carbohydrate and *vice versa.*

From the results reported by CAMPBELL, it would appear that accumulation of considerable quantities of nitrate in the leaves of annuals, biennials, and perennials whose above-ground portions mature and die before frost is of rather general occurrence in the period prior to blooming; but that the appearance of flowers and seed is accompanied by total or nearly total disappearance of nitrates from leaves and stems.

The work of SCHERTZ (**30**) on mottling of leaves of *Coleus,* taken in conjunction with the earlier work of SAMPSON (**29**), on abscission in the same plant, is of interest in this connection. The normal green leaves of this plant have a somewhat high content of nitrate at all stages of growth up to full size, no material reduction in nitrate content occurring so long as the leaf is functional. Leaves in which mottling had become perceptible were always lower in all forms of nitrogen (and especially in nitrate nitrogen) than normal green leaves; their phosphate content was only one-third that of normal leaves. In leaves in which the abscission layer was in process of formation, SAMPSON's analytical data indicate that there is a reduction in nitrate nitrogen in the leaf blade with a considerable increase in the immediate neighborhood of the abscission layer previous to leaf fall. There was little evidence of any general transport of carbohydrates, phosphates, or organic nitrogen compounds out of the leaf previous to its fall. SAMPSON gives no indication as to the age of his plants nor as to whether they had reached the flowering stage. The photographs accompanying the paper of SCHERTZ (**30,** figs. 5, 6) indicate that the plants from which leaves were taken were producing flowers. It would consequently appear that in *Coleus* no such reduction of nitrate content of the leaves occurs at flowering as was found by CAMPBELL in *Amaranthus* and other weeds.

NIGHTINGALE (**23**) concludes that "nitrates may be stored by the plant within itself until the proper conditions arise for synthesis to other forms of nitrogen." This is unquestionably true. The high nitrate content of young plants and the lower content of the mature plants examined by CAMPBELL may be due to exhaustion of available nitrates within reach of

the root system and to utilization of the stored nitrate in the construction of protein, or to the possession of high nitrate-absorbing capacity by the young plant and cessation of such absorption, regardless of supply, as its utilization in growth ceases after production of flowers and seeds.

Summarizing, it would appear that in annuals, nitrates are rather generally accumulated during the period of most active growth and exhausted during development of the reproductive structures.

Experimentation

The purpose of this paper is to record some of the findings in regard to the biochemical changes occurring in the rhubarb plant in the course of its seasonal growth. Particular attention has been directed to the changes in acidity, carbohydrate, and nitrogenous constituents occurring incidental to growth, maturity, and senescence of the aerial parts, and to the effects of the annual march of seasonal conditions upon the character of these changes.

Source of material

The material used in these studies of rhubarb was supplied through the kindness of D. N. Shoemaker and was grown in the variety test plot at Arlington Experimental Farm near Rosslyn, Va. The variety, Ruby, was originated at the Central Experimental Farm, Ottawa, Canada, and was named and distributed for trial by Dominion Horticulturist W. T. Macoun (**20**) in 1923. The planting was made in the spring of 1927 with material obtained by the usual method of dividing the crowns of old plants. The samples were taken in 1928, so that the planting was one year old. Generous applications of well rotted stable manure were made in 1927, but no manure or commercial fertilizer was applied in 1928. The plot was frequently cultivated throughout the season to kill weeds. No flower stalks developed upon any of the plants during the season. The variety is one that does not readily form flower stalks.

Methods of sampling

Four series of samples were taken during the season. The first of these series differed from the others in that it consisted of the first leaves which were developed by the plants in the spring. The plot was inspected on April 21, at which time the leaves were just appearing above ground. Identifying tags were attached to a considerable number of leaves having petioles 2–4 inches in length with the leaf blade not yet completely expanded. These leaves were arbitrarily assumed to be 10 days of age at this time. From the group of leaves so marked, samples were taken at intervals of 10 to 14 days, beginning April 21, up to June 15, at which time the leaves first marked were 65 days of age and were yellowing and dying.

From April 21 onward the plants were inspected at intervals of 5 to 8 days, and a number of developing leaves having petioles 2–4 inches long were tagged at each inspection. This made it possible to collect at one time a series of samples of leaves and petioles of known and varying ages up to senescence. Such collections were made on three dates, June 5, July 6, and October 17, each collection consisting of samples ranging in age from 5 to 8 days up to 60 to 65 days. In consequence, while the first series consisted of petioles and leaves all of which began their development at one time, the later series were made up of material beginning development at various dates throughout the entire period of active growth, from April 11 to October 9.

The samples were in all cases collected about 10 o'clock in the morning, after dew, if present, had evaporated. Each sample was made up of about 40 leaves from 20 plants, collected by pulling the petioles away from the crown, as is customary in harvesting for market. The material was immediately transferred to the laboratory and sampled for analysis as rapidly as possible, being protected from evaporation meanwhile. In taking the samples of leaf blades, the midrib and its lateral branches were removed and discarded, the sample consisting of mesophyll tissue with only the smaller veins, taken from various regions of a great number of leaves. The samples of petioles were prepared by cutting thin sections from upper, middle, and basal regions of each of a sufficient number of petioles to make up the desired quantity. Duplicate 100-gm. samples were taken in all cases. In taking the root samples, an entire plant was taken up, brought into the laboratory, and portions of the root system selected which were as nearly representative of the entire system as a whole as possible. As it was not possible to collect the very fine rootlets, the samples consisted chiefly of the thickened rootstocks and roots in which storage of starch and other substances occurs. The first root sample was taken April 21, at the outset of seasonal activities; the others were taken at the same time as the petiole and leaf samples.

Methods of analysis

The samples were preserved in sufficient 90 per cent. alcohol to make the final concentration 75 per cent. and were heated to boiling to stop enzyme action. The following November the samples were extracted with alcohol, the extracts made up to volume, and aliquots taken for determination of soluble solids, sugars, tannins, and nitrate nitrogen. Acid-hydrolyzable polysaccharides were determined upon a portion of the insoluble residue. Total nitrogen determinations were made upon portions of material dried in an air oven at 70° C. and finished in a vacuum oven.

Sugars.—Determinations were made before and after hydrolysis with HCl at 70° F. by the Munson-Walker method, the cuprous oxide being

determined by the volumetric permanganate method. The results for free reducing sugars are omitted from the tables.

TITRATABLE ACIDITY.—Titrations were made with N/10 NaOH against phenolphthalein and the results expressed as malic acid.

TANNINS.—Titrations were made with N/20 $KMnO_4$ with indigo-carmine as indicator.

ACID-HYDROLYZABLE POLYSACCHARIDES.—The official method of the Association of Official Agricultural Chemists for determination of starch by direct acid hydrolysis was employed. (See footnote, p. 462.)

TOTAL NITROGEN.—Determinations were made by the KJELDAHL method as modified to include nitrate nitrogen.

NITRATES.—The method recommended by the Official Methods for determination of nitrates in meats was followed.

AMINO ACIDS.—Determinations were made by the VAN SLYKE method upon aliquots of the material after preservation in alcohol. In view of the work of WEBSTER (**37**), it is doubtful whether the results can be considered reliable, but they may have a certain degree of comparative value.

WEATHER CONDITIONS DURING SAMPLING PERIOD

The weather conditions for the 12 months of 1928 are given in table I, which summarizes for each month the rainfall, normal mean temperature, and percentage of the possible sunshine, with departures from the respective monthly normals. The year was somewhat warmer than normal up to April 1, with somewhat deficient rainfall. April had a moderate excess of rain and was below normal in temperature; May was very close to normal in all respects; June and July were deficient in rainfall and drought conditions had become well established by August 11 and 12, at which time rainfall of more than 8 inches occurred, followed by several further rains which brought the total for the month up to 14.41 inches. September was markedly cool and deficient in sunshine; October was dry and warm, as were the following months.

In table II the weather conditions for 40 days preceding the taking of each sample are stated by periods of 10 days each, mean temperature, percentage of possible sunshine, and rainfall having been calculated from the daily records of the Weather Bureau. Certain facts are immediately obvious from inspection of the table.

The first series of samples began development about April 10 and were collected at various times up to June 15. The second series began development at various times between April 21 and June 1 and were taken all at one time on June 5. The period from April 21 to June 15 was one of progressively rising temperature, with sunshine close to the normal and with no marked deficiencies in rainfall. The 10-day period ending April

TABLE I

MONTHLY PRECIPITATION, MEAN TEMPERATURE, AND PERCENTAGE OF POSSIBLE SUNSHINE, WITH NORMALS AND DEPARTURES THEREFROM, FOR THE YEAR 1928; WASHINGTON, D. C.

MONTH	RAIN	NORMAL	DIFFERENCE	TEMPERATURE	NORMAL	DIFFERENCE	SUNSHINE	NORMAL	DIFFERENCE
	inches	*inches*	*inches*	*°F.*	*°F.*	*°F.*	*per cent.*	*per cent.*	*per cent.*
Jan.	2.63	3.38	− 0.75	35.9	33.4	+2.5	65	46	+19
Feb.	2.69	3.42	− 0.73	37.7	35.3	+2.4	58	54	+ 4
Mar.	2.17	3.85	− 1.68	44.1	42.6	+1.5	60	51	+ 9
April	4.49	3.25	+ 1.24	51.9	53.3	−1.4	55	58	− 3
May	4.00	3.83	+ 0.17	63.1	63.7	−0.6	62	61	+ 1
June	2.66	4.13	− 1.47	71.0	72.2	−1.2	53	62	− 9
July	2.17	4.71	− 2.54	78.2	76.7	+1.5	66	64	+ 2
Aug.	14.41	4.01	+10.40	77.0	75.0	+2.0	49	61	−12
Sept.	4.29	3.24	+ 1.05	64.9	68.1	−3.2	43	63	−20
Oct.	0.67	2.84	− 2.17	59.9	57.4	+2.5	61	61	− 0
Nov.	2.00	2.37	− 0.37	49.4	45.2	+4.2	48	56	− 8
Dec.	1.21	3.32	− 2.11	39.2	36.6	+2.6	58	51	+ 7

TABLE II

WEATHER CONDITIONS PRECEDING THE TAKING OF SAMPLES BY 10-DAY PERIODS

SAMPLING	1ST 10 DAYS PRECEDING SAMPLING			2ND 10 DAYS PRECEDING SAMPLING			3RD 10 DAYS PRECEDING SAMPLING			4TH 10 DAYS PRECEDING SAMPLING		
	MEAN TEMP.	SUNSHINE	RAIN	MEAN TEMP.	SUNSHINE	RAIN	MEAN TEMP.	SUNSHINE	RAIN	MEAN TEMP.	SUNSHINE	RAIN
	°F.	*per cent.*	*inches*	°F.	*per cent.*	*inches*	°F.	*per cent.*	*inches*	°F.	*per cent.*	*inches*
Series I:												
April 21	51.4	57.9	0.24	55.1	61.3	1.42	52.3	59.9	0.35	52.9	59.7	2.01
May 5	58.3	66.4	1.96	49.7	54.9	1.11	53.9	48.3	1.32	53.9	56.5	4.39
May 19	64.2	70.6	1.01	61.6	57.2	1.28	48.5	44.3	3.02	58.1	57.4	5.31
May 26	65.5	45.4	2.54	58.7	72.7	1.35	59.1	57.2	2.00	61.1	61.3	3.72
June 5	65.6	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
June 15	69.9	56.8	0.49	64.6	54.0	0.37	65.5	46.4	2.54	66.6	52.4	3.40
Series II:												
June 5	65.5	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
Series III:												
July 6	76.3	75.7	0.55	71.8	42.9	1.25	69.2	60.2	0.49	72.4	59.6	2.29
Series IV:												
Oct. 17	67.0	61.1	0.00	59.8	51.4	0.55	74.0	64.7	0.00	67.0	59.1	0.55

21 had only 0.24 inch rainfall, but the preceding 10 days had 1.42 inches. The period May 26 to June 15 had only 0.86 inch, but the preceding 10-day period had 2.54 inches. In no case during the taking of these samples was there a period of drought such that the soil became markedly deficient in moisture, nor was there any such marked or prolonged departure from normal in temperature or in incidence of sunshine as might be expected appreciably to affect the rate of development of the plants.

The third series, collected July 6, presents some contrasts with the second series. The mean temperature for the 30 days preceding July 6 was 9 degrees higher than for the corresponding period ending June 5, while the rainfall was only 54 per cent. of the amount for that period. These samples consequently developed in a period that combined high temperatures and resulting maximum transpiration with a considerable deficiency in water supply. The fourth series, collected October 17, developed in a period of markedly deficient precipitation. The 10 days immediately preceding sampling were entirely rainless, the second 10 days had 0.55 inch, and the third 10-day period was again rainless. The dry period had been of sufficient duration at the time of sampling to reduce soil moisture to a very low level, and the mean temperature for the month preceding sampling was only 5.4 degrees below that for the third series and 3.7 degrees above that of the second series.

Summarizing, the first and second series developed in a period of ample rainfall and low mean temperature; the third series developed in a period of subnormal rainfall and maximum mean temperature; the fourth series developed in a period of markedly deficient rainfall and intermediate mean temperature. The percentage of possible sunshine is markedly uniform in all the series. Obviously, any effect of environmental conditions apparent in comparing the several series may be expected to be that of progressive reduction of moisture content of the soil accompanying the progress of the season.

Analytical results

The results of the analyses are assembled in table III, and the changes in the several constituents are graphically presented in figures 1 to 5. The general course of the changes in composition of the petiole and leaf will be indicated, after which the details of differences between the several series will be discussed.

Total solids

It is noted at once that there was a very striking difference in total solids between the mesophyll tissue and the petioles. The total solids of the leaf tissue were everywhere much greater, in the early stages about three times as great, as in the petiole. Total solids of the leaf decreased

TABLE III

COMPOSITION OF RHUBARB PETIOLES AND LEAVES AT DIFFERENT STAGES OF DEVELOPMENT IN PERCENTAGE OF FRESH GREEN WEIGHT

Date of sampling	Age in days	Size of leaves in inches		Total solids		Solids soluble in alcohol		Alcohol-insoluble residue		Total sugar	
		Length petiole	Width leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf
Series I:											
April 21	10	2– 4	3– 4	5.21	15.49	2.96	5.64	2.25	9.85	0.65	0.74
May 5	24	10–12	8–10	4.80	12.97	2.44	4.52	2.36	8.45	0.30	0.62
May 19	38	12–14	10–12	5.71	10.02	2.80	3.08	2.91	6.94	0.42	0.42
May 26	45	14–18	12–14	5.87	9.71	2.90	3.32	2.97	6.39	0.60	0.47
June 5	55	14–18	12–14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56
June 15	65	14–18	12–14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48
Series II:											
June 5	5	3– 4	3– 4	5.33	16.40	2.84	5.60	2.49	10.80	0.40	0.89
" "	8	6–10	5– 7	4.83	14.63	2.60	5.40	2.23	9.23	0.39	0.70
" "	11	10–12	6– 8	4.60	14.59	2.48	4.84	2.12	9.75	0.33	0.60
" "	15	12–14	8– 9	4.84	12.65	2.72	4.08	2.12	8.59	0.39	0.62
" "	19	14–16	8–10	4.56	13.27	2.56	4.16	2.00	9.11	0.29	0.40
" "	25	16–18	14–16	5.25	12.74	2.96	3.74	2.28	9.00	0.52	0.76
" "	32	18–21	14–16	5.81	11.17	3.16	3.28	2.65	7.89	0.60	0.65
" "	40	18–21	14–16	6.17	11.62	3.37	3.32	2.80	8.30	0.90	0.51
" "	55	14–18	12–14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56
" 15	65	14–18	12–14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48
Series III:											
July 6	5	4– 6	3– 4	6.57	18.07	3.44	5.80	3.13	12.27	0.40	0.79
" "	6	10–12	5– 7	6.45	17.59	3.44	6.28	3.01	11.31	0.50	0.71
" "	13	12–16	7–10	6.15	15.82	3.20	5.40	2.95	10.32	0.60	0.61
" "	28	18–24	18–22	7.19	13.04	3.60	4.16	3.59	9.88	1.05	0.74
" "	42	20–28	17–22	7.43	12.37	3.48	3.44	3.95	8.93	0.91	0.52
" "	60	20–28	17–22	6.82	10.06	2.96	2.76	3.86	7.30	0.56	0.42
Series IV:											
Oct. 17	8	9–14	7–10	6.29	15.57	3.56	5.32	2.73	10.25	1.04	0.74
" "	12	14–18	10–12	6.35	15.02	3.44	5.32	2.91	9.70	1.04	0.66
" "	24	16–20	10–14	7.22	13.34	3.76	4.32	3.46	9.02	1.14	0.61
" "	40	16–20	10–14	7.23	13.33	3.84	4.24	3.39	9.09	1.05	1.36
" "	60	16–20	10–14	5.64	10.00	2.52	3.20	3.12	6.80	0.58	0.61
						Root					
April 21				13.14		5.96		7.18		1.76	
May 19				12.08		5.68		6.40		1.14	
July 6				22.09		6.76		15.33		2.44	
Oct. 17				24.44		6.40		18.04		1.69	

TABLE III—(*Continued*)

	Acid-hydrolyzable polysaccharides		Acid as malic		Tannin		Nitrate nitrogen		Amino nitrogen		Total nitrogen	
	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf
May 19	0.55	0.90	0.999	0.441	0.096	1.08	0.014	0.002	0.045	0.042	0.211	0.943
July 6	0.53	0.79	0.948	0.688	0.058	0.432	0.030	0.004	0.029	0.039	0.203	0.824
Oct. 17	0.67	0.69	0.884	0.595	0.086	0.277	0.067	0.007	0.027	0.032	0.178	0.560
	0.68	0.59	1.000	0.611	0.109	0.326	0.065	0.007	0.036	0.038	0.155	0.409
	0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
	0.70	0.62	0.749	0.540	0.110	0.236	0.100	0.017	0.020	0.035	0.130	0.435
	0.54	0.95	1.080	0.593	0.122	0.774	0.024	0.003	0.043	0.062	0.198	0.964
	0.48	0.89	1.110	0.652	0.109	0.581	0.029	0.005	0.045	0.061	0.160	0.912
	0.46	1.05	1.080	0.639	0.127	0.487	0.030	0.005	0.055	0.054	0.136	0.895
	0.47	1.06	1.110	0.748	0.096	0.338	0.032	0.005	0.040	0.032	0.142	0.813
	0.42	1.21	1.070	0.785	0.088	0.348	0.035	0.005	0.039	0.050	0.149	0.741
	0.45	1.06	1.085	0.704	0.091	0.335	0.038	0.006	0.030	0.038	0.127	0.727
	0.58	0.92	1.140	0.715	0.087	0.249	0.044	0.009	0.030	0.028	0.134	0.628
	0.72	0.99	1.160	0.713	0.079	0.282	0.056	0.009	0.026	0.032	0.130	0.531
	0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
	0.70	0.62	0.749	0.540	0.110	0.236	0.099	0.017	0.020	0.035	0.130	0.435
	0.76	1.06	1.520	0.749	0.168	1.222	0.017	0.002	0.071	0.093	0.267	1.090
	0.79	1.30	1.580	0.821	0.153	0.975	0.022	0.002	0.068	0.101	0.228	0.944
	0.77	1.78	1.380	0.799	0.123	0.455	0.026	0.003	0.057	0.065	0.197	0.867
	0.84	1.10	1.320	0.843	0.201	0.437	0.027	0.003	0.043	0.058	0.149	0.662
	0.78	0.81	1.150	0.628	0.162	0.290	0.053	0.005	0.038	0.055	0.148	0.594
	0.75	0.75	0.781	0.493	0.265	0.265	0.073	0.010	0.010	0.043	0.121	0.316
	0.63	0.98	1.560	0.893	0.111	0.359	0.023	0.003	0.070	0.066	0.201	0.807
	0.64	0.84	1.430	0.816	0.114	0.385	0.026	0.003	0.070	0.055	0.186	0.734
	0.74	0.90	1.300	0.876	0.110	0.392	0.026	0.003	0.050	0.072	0.180	0.675
	0.72	0.82	1.280	0.950	0.115	0.385	0.030	0.004	0.041	0.041	0.159	0.635
	0.67	0.65	0.934	0.680	0.100	0.331	0.045	0.005	0.039	0.046	0.132	0.367
						Root						
	2.24		0.362		1.260		0.0082		0.096		0.417	
	1.91		0.717		1.410		0.027		0.069		0.386	
	10.41		0.560		1.460		0.022		0.054		0.279	
	12.60		0.576		1.500		0.026		0.062		0.447	

progressively with increasing age until the leaf had practically ceased to grow, then fell off more slowly until the leaf was senescent (fig. 1, curves *A'–D'*). The whole course of development of the leaf is accompanied by a progressive hydration which varies in rate with the amount of external water supply, as evidenced by the flatness of curve *D'* (representing a series grown under conditions of rather severe drought). It would appear that the developing leaf is high in its content of hydrophilic colloids and that these become progressively saturated as the leaf ages. With attainment of full size and cessation of the formation of new colloidal materials, absorption of water slows down, but continues, except in one case (figure 1, *A'*), up to the final sampling. It seems possible that the old leaf may die because it can no longer obtain water, lacking a saturation deficit of sufficient magnitude to maintain a water balance against transpiration.

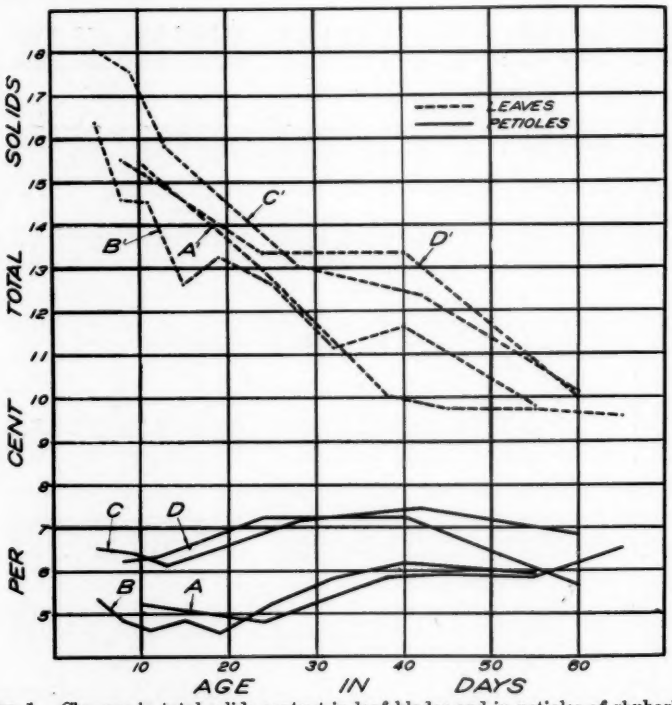

FIG. 1. Changes in total solids content in leaf blades and in petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

The changes in total solids content of the petioles take a definite course which is of interest (fig. 1, *A–D*). During the period of rapid elongation of the petiole the solids decrease. As elongation slows down and the period of maximum photosynthetic activity of the leaf is entered upon, solids increase again, becoming stationary or falling off somewhat as senility of the leaf commences.

It will be observed that there is considerable difference in total solids content of both leaves and petioles in the series taken at different seasons. In series I and II, developing in the spring, total solids are consistently lower at all stages in both leaves and petioles than in series III and IV, collected in July and October. Further, series IV, collected in October, is distinctly higher in solids than series III, except in the old petioles. These differences would appear to be explained by the fact that the water supplied by rainfall during the last month of development of series III and IV was only about one-half and one-eighth that received in the corresponding period by series I and II. It would appear that the saturation deficit of the leaf tissues is largely satisfied in the first half of the life period if the water supplied the root is ample, but that its satisfaction is progressively retarded with progressive decrease in water supply.

Total solids of the root show a decrease from the first sampling, April 21, to that on May 19, due to depletion of the reserves of the root system by the developing foliage. On July 6, storage of reserves is well advanced, and on October 17 the total solids content of the roots is more than 200 per cent. of that present at the height of the growing period on May 19.

Alcohol-soluble solids

The alcohol-soluble solids of the leaf blade are high in the earliest samples taken and decrease rapidly until the leaf is full-grown, then more slowly up to old age. The final value in old leaves is about 50 per cent. of that of the youngest samples. In the petioles, soluble solids fluctuate within narrow limits, decreasing somewhat in the period of most rapid growth, then returning to the original level. In series III and IV there is a decrease in the final sample not found in the earlier series, for which no explanation is apparent.

The general level of alcohol-soluble solids is higher in the petioles of series III and IV, but there is no consistent difference in the leaves. The alcohol-soluble solids of the root show little change, decreasing slightly from April to May, then rising to somewhat higher levels in July and October.

Alcohol-insoluble solids

The alcohol-insoluble solids of the leaf blade decrease rather rapidly until the leaf is full-grown, then more slowly, with some fluctuations, to

a value at final sampling about two-thirds of that found in the earliest sample. The alcohol-insoluble fraction in the petioles remains stationary or decreases during rapid growth, then rises somewhat with increasing age. The general level of insoluble solids in the petioles is a little higher in series III and IV than in the earlier series.

The fluctuations in both alcohol-soluble and alcohol-insoluble fractions in petioles and leaves of series I suggest that both may be temporarily affected by climatic conditions, and particularly by rainfall.

Alcohol-insoluble solids in the roots decrease to a minimum in May, when rapid development of aerial parts at the expense of stored reserves is occurring, then increase rapidly to the July sampling, and more slowly to October, when the amount is 282 per cent. of that present in mid-May. The large seasonal change in total solids of the roots is practically wholly due to the change in amount of the alcohol-insoluble fraction.

Sugars

It is obvious that the total sugars are low in both leaf and petiole, and the differences between them are not marked. There is a slight tendency for the sugars of the leaf blades to be highest in young samples and lowest in old samples, while lowest in young petioles and higher in older ones; but between the two extremes there is considerable and rather abrupt fluctuation, so that the curves are irregular. It does not seem to be justifiable to draw any very definite conclusion as to the mechanism of the transportation and the use that is made of the sugars.

Acid-hydrolyzable substances (polysaccharides)[1]

The acid-hydrolyzable substances are very low in both the leaf and petiole. The figures indicate that the change in content with age is not great. They seem to be highest in both petioles and leaves in series III. In the series taken in June and July these substances are highest in the samples of medium age, being low when young and decreasing again with age. This is not apparent in the other two series. Starch is practically absent in the petiole and not high in the leaf. Acid-hydrolyzable substances are at a minimum in the root in May, increasing to over 600 per cent. of

[1] The term "acid-hydrolyzable polysaccharides" as applied to the substances converted to reducing sugars by the official method of direct acid hydrolysis for starch of the Association of Official Agricultural Chemists, which was here used, is technically inaccurate, since the reducing sugars obtained may be derived from polysaccharides, pentosans, glucosides, nucleic acids, or other substances having a sugar group in the molecule. While a misnomer, it has the merit that prolonged usage has made biochemists familiar with its real meaning as here employed. "Acid-hydrolyzable substances," while less restrictive, is indefinite unless the exact conditions of the hydrolysis are stated.

the spring value in October. Approximately one-half of the total solids of the October sample is in this fraction.

Tannin

There is a striking difference in the tannin content of the leaf and petiole, that of the leaf being one and one-half to four times as high as that of the petiole. In the petiole the stage of maturity makes no striking difference until the very old samples are reached, when it becomes much lower. In the leaf the tannin is highest in the young and lowest in the old material. The decrease in amount in the leaf is rapid in the period of most rapid growth, 5 to 20 days of age, then becomes slow and fluctuates irregularly. Tannins in the root increase slightly from first to last sampling.

Titratable acidity

The titratable acidities of petioles and leaf blades show consistent and striking differences at all stages (fig. 2). That of the petiole is always much higher, being double that of the blades in the youngest samples and about one and one-half times that of the blades in the older ones. The changes

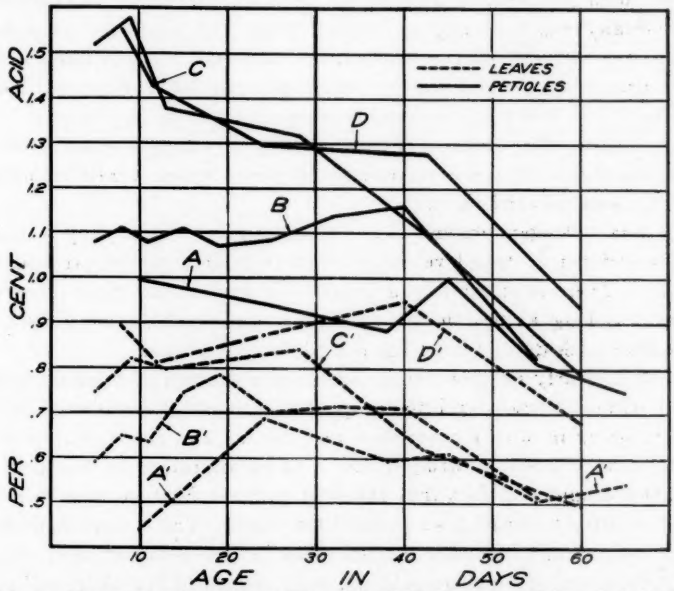

FIG. 2. Changes in titratable acidity of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

with age in the leaf tissue are an increase in the period of rapid growth, a period of little change during the period of maximum photosynthetic activity of the leaf, and a gradual decline as the leaf becomes old. In the petiole, acidity remains somewhat constant or decreases only slightly until the petioles are 45–55 days old, then declines rather rapidly in the old samples. It is noteworthy that the acidity of both petioles and blades of series III and IV is considerably higher throughout than in series I and II, that of the petioles being about 150 per cent. This difference may find its explanation in the reduction of available water supply during the development of the later series.

Total nitrogen

The curves representing total nitrogen content of petioles and leaves show the striking differences which exist (fig. 3). The total nitrogen of the leaf mesophyll is in the young leaf 400 to 500 per cent. of that of the petioles of like age; that of the old leaves is approximately 300 per cent. of that of old petioles. There is a marked decrease in total nitrogen of leaves with age, the final values for leaves 60 to 65 days old ranging from 35 to 45 per cent. of those of the youngest samples. The decrease is not especially rapid at any particular stage, but continues at a rather uniform rate throughout the whole life of the leaf. The petioles also decrease progressively and rather uniformly in total nitrogen content throughout life, the total decrease amounting to 30 to 50 per cent. of the initial content. The differences in level of total nitrogen content between the several series are not great in amount and are not correlated with the march of seasonal conditions.

The total nitrogen content of the roots decreases up to July 6, but has risen on October 17 to a level slightly above that of the initial sample of April 21. There is apparently a progressive depletion of total nitrogen in the roots so long as new leaves are being developed, followed by an increase after such development has ceased for the season.

There is clearly no such great and rapid reduction of total nitrogen in the old leaves by transfer into the perennial parts as has generally been found to occur in woody perennials and trees. The curve of decrease in the leaf is very nearly a straight line with no alteration in direction with old leaves, despite the fact that the blades of the final samples were generally yellowing and had dead areas at the edges. The present data do not permit decision as to whether the reduction in percentage of total nitrogen is only apparent and not actual, and attributable to the changes in composition occurring with age, or whether slight actual decrease occurs.

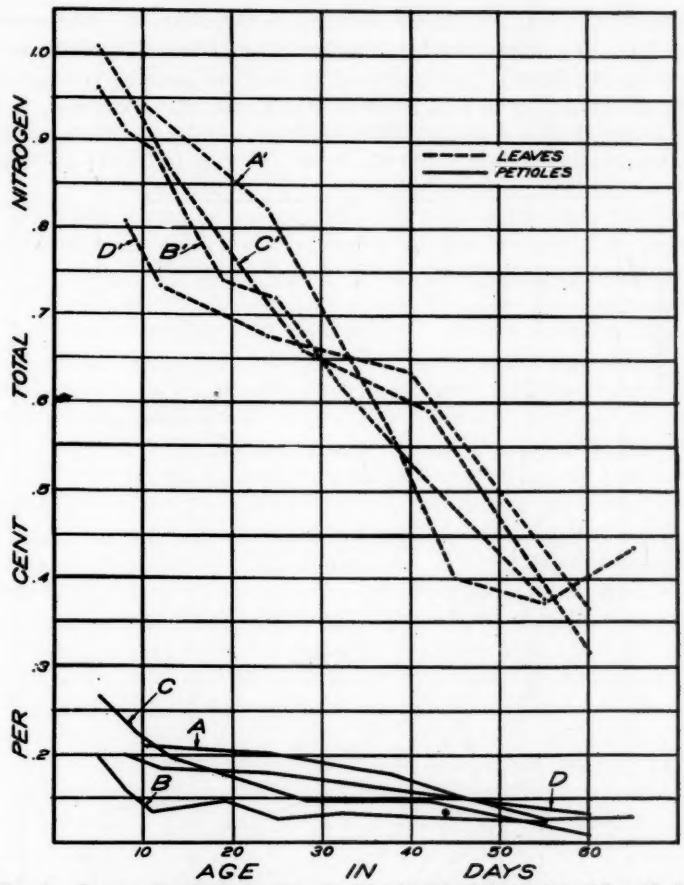

FIG. 3. Changes in total nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*. *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

AMINO NITROGEN

The determinations of amino nitrogen were made upon material preserved in alcohol. They were completed prior to the appearance of the paper by WEBSTER (37) on the losses of amino nitrogen occurring in material preserved in this manner, especially when nitrates are present. It is very questionable whether the results have any quantitative value. The extremely irregular character of the curves is not conducive to confidence in

their accuracy. The results are included in the table and represented in figure 4 for any comparative value they may have. They indicate that there is a progressive but rather irregular decrease in amino nitrogen from first to last samplings in both leaf blades and petioles. They seem to indicate also that there is a decided difference in general level of amino nitrogen content between the earlier and the later series, as series III and IV are

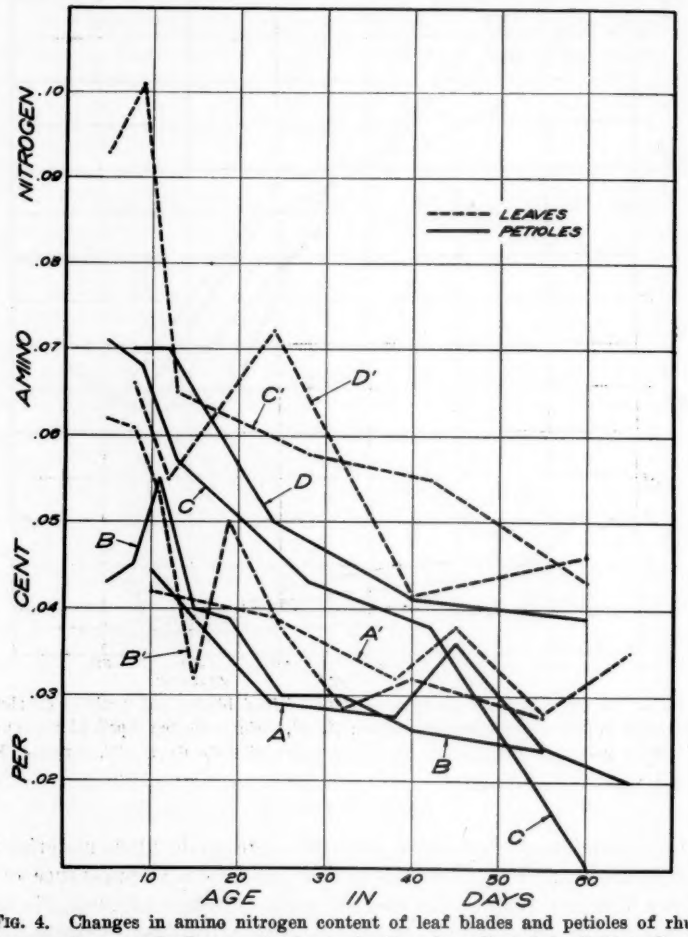

FIG. 4. Changes in amino nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 5; *D*, *D'*, series collected October 17.

very materially higher throughout, both in petioles and leaves, than are series I and II. Whether the concurrence of higher amino nitrogen values in these series with the lower available water supply during their development is significant or merely a coincidence cannot be determined in view of the questionable character of the method employed.

Nitrate nitrogen

As has been the case with several other constituents, and particularly with total nitrogen, there are striking differences between the leaf mesophyll and the petiole in nitrate nitrogen content. In contrast to total nitrogen, nitrate nitrogen is highest in the petioles, and the differences between petioles and leaves are greatest in the oldest material. In both petioles and leaves, nitrate nitrogen is minimum in amount in the youngest samples and rises with age (fig. 5). The amounts present in leaves are always low but the increases, while small, are consistent and significant, the final samples of the several series having amounts ranging from 160 to 850 per cent. of those of the initial samples of their respective series. The amounts initially present in the youngest petioles are much larger than in leaves, and increase rapidly with age, the final content of the old petioles ranging between six and nine times that of the old leaf tissues. It is apparent that the plant absorbs quantities of nitrate nitrogen far in excess of its anabolic requirements, and that the petioles serve as storage organs for the deposition of the excess. That this nitrate nitrogen is neither used in the leaf nor returned to the roots is clear, since the petiole attains its maximum nitrate nitrogen content when the leaf blade is yellowing and dying. The nitrate nitrogen content increases rather rapidly, even during the period of rapid growth when synthesis of protein is actively in progress, but becomes more rapid after the leaves are full-grown. There is, of course, a possibility that lack of some other constituent prevented utilization of the nitrate nitrogen and that it was consequently stored in the petioles, the metabolic processes never becoming such that it could be used. Further study will be necessary to determine whether development of flower stalks is accompanied by withdrawal of nitrate nitrogen from the petioles, since the variety employed is one which only exceptionally develops flower stalks, and none were developed during the progress of the work.

The progressive accumulation of nitrate nitrogen in the petioles is a matter of great practical importance in the canning of rhubarb, since nitrate nitrogen has an intense corrosive action upon the tin can. This matter has been dealt with in detail by Culpepper and Moon (**14**) in their report upon the behavior of rhubarb in canning.

It is unfortunate that soil samples were not taken and that it cannot be determined whether the amount of nitrate nitrogen in the tissues shows any

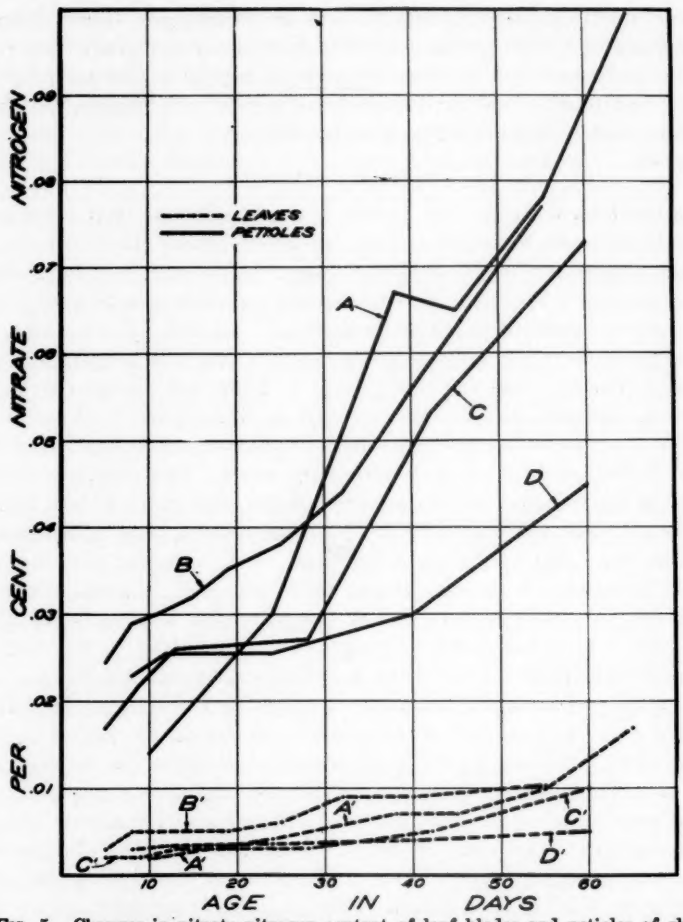

FIG. 5. Changes in nitrate nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

correlation with the amount present in the soil. It is probable that the reduction in amount of available soil nitrates with advance of the season may be responsible for the differences between the earlier series and series III and IV. In the first two series, nitrates are higher in both leaf blades and petioles than in the later series. Series III, taken in July, is consistently somewhat lower throughout in both leaves and petioles than I and

II while series IV is lower than III, the difference being especially marked in the old petioles. This may be a result of progressive decrease of available nitrate in the soil. Another fact which is significant in relation to the low nitrate content of series IV is the extraordinarily heavy rainfall of August, which had a total precipitation of 14.41 inches, or 10.40 inches' excess over the normal, largely occurring as heavy rains between August 11 and August 26. This excessive precipitation, which was followed by drought extending from mid-September through October, probably resulted in leaching out much of the nitrates present in the soil, and is without doubt a significant factor in the reduction of the nitrate content of the older samples of the October series to less than half that of the corresponding samples taken earlier in the season. That the reduction of available nitrate was limited in its effect to reducing the storage of excess nitrate in the petioles and did not affect the total nitrogen content of the leaves and petioles is immediately apparent from the graph (fig. 3, curves *D* and *D'*).

Discussion

The biochemistry of the rhubarb plant presents some interesting points of difference from that of woody perennials or that of annuals and biennials, which may be briefly summarized.

The work of LeClerc du Sablon (**15**) with leaves of chestnut, pear, peach, quince, and willow trees and raspberries, and that of Tucker and Tollens (**34**) with *Platanus occidentalis,* indicate that in these forms there is a progressive decrease in water content with age. A similar situation prevails in annuals, and is attributable in part to progressive increase in vascular elements and cutin and in part to the presence of such products of photosynthesis as starches and sugars. In the work of the investigators cited, as well as in all similar studies found in literature, the entire leaf and petiole were taken together, so that it is impossible to ascertain the nature of the changes taking place in the leaf mesophyll as distinguished from petiole, midrib, and veins.

In the rhubarb leaf from which petiole and larger veins have been separated, the situation with respect to water content is precisely the reverse of that recorded for entire leaves of other plants. The youngest material obtained had a minimum water content which increased steadily with advance in age up to senescence, but most rapidly during the period of rapid increase in size. In this respect the growth of the leaf blade of rhubarb bears less resemblance to that of other and entire leaves than it does to that of young fruits, which have been shown by Caldwell (**11**) to be initially high in total solids and to increase markedly in water content during the phase of most rapid growth. The extent to which the situation

found in the leaf blade actually differs from that in the mesophyll of other types of leaves can be known only when the changes in the leaf blade proper, separated from the petiole and principal veins, have been studied in such leaves. In one respect the rhubarb leaf differs markedly from those of most other dicotyledonous plants, namely, in the practical absence of stored starch at all times.

The carbohydrate economy of the rhubarb plant presents some interesting problems. The total sugar content of the leaf is always very low, reaching 1 per cent. of the fresh weight in only one sample. It is with one exception highest in the youngest leaf blades of the series, collected while still largely folded and partially covered by the soil, and consequently before active photosynthesis could have begun. The amount present falls off slightly during the period of most rapid growth, rises as the leaf becomes full-grown, and drops again in senescence; but the changes are within extremely narrow limits and could not be considered significant did they not occur in all the series. The sugar content of the petiole is also low at all stages of growth, being sometimes above and sometimes below that of the corresponding leaf blade. In the October series it is somewhat higher throughout than in the earlier series, but drops abruptly to the same general level in the final sample. The sugar content of the root system is practically the same on April 21 and October 17, but is lower in May and higher in July.

Starch is present only in traces in petiole and leaf, as determined by microchemical tests, and the total acid-hydrolyzable substances are always low. In the leaf the amount of such substances present in the young, partially folded blade is in most cases about as great as in the fully expanded photosynthetically active leaf, and the amount present in the old, yellowing leaves is two-thirds to three-fourths that present in the active leaves. The acid-hydrolyzable substances of the petiole are lower in amount than those of the leaf blades, and fluctuate irregularly without significant change from youngest to oldest samples. There is no evidence of an accumulation of starch either in leaf or petiole as a result of photosynthetic activity, and the results indicate that there is no accumulation of sugar as such in the leaf. The amounts of sugar in the leaves and their petioles vary independently in such fashion as to make it clear that there is no concentration gradient such as has been shown to exist in various other plants. While the samples were uniformly collected at 10 A. M. on clear days, when photosynthesis should have been actively in progress for several hours, the leaves and petioles of fully functional leaves show no consistently higher content of sugar than do the young, not yet expanded leaves or the old, yellowing ones. The manufacture and transfer of photosynthetic products is accom-

plished without any such accumulation of sugars in the leaf or in the conducting system as is a normal accompaniment of photosynthesis in foliar organs. The absence of evidence for such accumulation is the more remarkable in view of the rather high rate of increase in dry weight found by SACHS (**28**) in *Rheum*, by MÜLLER (**21**) in the related *Rumex*, and by BROWN (**10**) in *Polygonum* when the leaves were exposed to bright illumination.

The acid-hydrolyzable substances of the roots were low when first examined on April 21, at which time the largest leaves had petioles 2–4 inches in length and had only partially unfolded their blades. The content was slightly lower a month later. On July 6 it had increased to 545 per cent. and on October 17 to 659 per cent. of the amount found on May 19. The concurrent changes in sugar content of the roots are within rather narrow limits, and the variations in total solids agree closely in direction and amount with the changes in hydrolyzable substances.

In contrast with the low concentration of sugars in petiole and leaf and the absence of a concentration gradient in sugars from leaf blade to petiole, the acid content of the leaf mesophyll is always markedly below that of the petiole. Also, the acidity of both leaf blade and petiole increases to a maximum as the leaf attains full size and declines markedly as the leaf becomes senescent. In both blade and petiole the acid content at all stages of growth equals or exceeds the sugar content, and in the petioles is often two or three times as great. The acid content of the root system fluctuates considerably, being lowest in April, increasing 100 per cent. in the next month, and then declining to a nearly constant level from July through October. At all times the acidity of the roots is below that of the leaves and half or less than half that of the petioles. Since NELSON (**22**) found 1.77 per cent. malic, 0.41 per cent. citric, and 0.12 per cent. oxalic acid in the petioles, the results of the acidity determinations are reported as malic acid.

In the present work, only such oxalates as are soluble in both 80 per cent. alcohol and in water are included in the determinations of titratable acidity, as the titrations were made upon aliquots of the alcoholic extract after evaporating the alcohol and taking up in water. This method includes free acids and such soluble oxalates as those of potassium, sodium, and magnesium, but does not include calcium oxalate. According to ANGERHAUSEN (**1, 2**), the oxalates of the leaf blade of rhubarb are largely water-soluble; those of the petiole practically wholly calcium oxalate. The data of table III indicate that total titratable acidity is at all times much higher in the petioles than in the leaf parenchyma, but give no indication as to the nature of the acid or acid salts present. No determinations of the total oxalate content of leaf blades, petioles, or roots were made.

The fact that the acid content of the young petiole and leaf is already rather high at a time when the leaves have barely emerged above ground and the blades have only partially expanded raises some interesting questions as to the rôle played by acids in the economy of the plant. CALDWELL (**11**) found that the period of most rapid growth in young fruits was also a period of rapid increase in both actual and titratable acidity, both of which culminated in maximum values about the time that the curve of growth passed into the self-retarding phase. His results indicate that in fruits, maximum acid production accompanies rapid cell division and increase in size, and a similar situation appears to exist in the young leaf.

Since the acid content of the mesophyll tissue is consistently lower than that of the petiole, the question arises whether acids are formed in the mesophyll directly from the products of photosynthesis and transferred into the petioles, or whether they are formed in the petioles as well as in the leaf tissue. STEINMANN (**32**) found that the titratable acidity of the mesophyll tissues of rhubarb increased slightly (10–15 per cent.) from apex to base of the leaf, while the increase in the veins from the smaller veinlets to the basal portion of the midrib was about 60 per cent., and a further slight increase was found in the petiole. These results STEINMANN considers as evidence that acids are formed in the leaf and transported to the roots through the petioles in the same manner as carbohydrates. The increase in acidity occurring in the leaf during a day's exposure to sunlight and the progressive decrease occurring in leaves kept in darkness in his experiments support his conclusion. His most conclusive proofs that acids are formed in the leaf and transported through the stem are derived from a series of experiments in which the petiole was cut half way through at the base of the leaf, thus severing the vessels of one-half the blade. After exposure to a day's sun, the acidity of the intact half of the leaf was always lower than that of the half with severed vessels; further, a reduction in acidity during darkness occurred in the intact half while no change occurred in the severed half. The transport of acids is consequently a constant process, in light as in darkness. STEINMANN also points out the possibility that organic acids may be not products of disintegration processes but intermediate products of constructive assimilatory processes. RUHLAND and WETZEL (**27**), on the other hand, consider that the progressive accumulation of acids and of ammonia and nitrate nitrogen in the plant with increasing age is the result of deamination of proteins.

ANGERHAUSEN (**1, 2**) states that the oxalic acid content of the leaf blades is approximately double that of the petioles, and that the oxalates of the leaf are predominantly potassium oxalate while calcium oxalate is predominant in the petiole. RUHLAND and WETZEL state that the youngest petioles contain only succinic and malic acid with scarcely a trace of oxalic,

and that later in their development there is a sharp decrease in the former concurrently with rapid increase in oxalic acid. The work of VICKERY and PUCHER (**36**), and that of SMIRNOW, ERYGIN, DRBOGLOW, and MASCHKOWZEN (**31**), indicates that the situation in tobacco is the reverse of that in rhubarb, as oxalic acid is present in the young tobacco plant but decreases as development of the plant proceeds.

BERTHELOT and ANDRÉ (**5**) determined the oxalic acid content in roots, stems, petioles, and leaves of *Rumex acetosa* in young, actively growing and old, fruiting plants (June 26 and September 27). In the young plant the oxalic acid content of the leaf blades exceeded that of the petioles and larger veins in the ratio of 12 to 10; in the old plants the acid content of leaf blades was lower than that of petioles and stems, the ratio being 4 to 5. The acid of the leaf blades was predominantly in soluble form in both young and old plants; that of the petioles was predominantly soluble in young plants and chiefly insoluble in old ones. The acid of the roots was wholly in insoluble form. The relative amounts present in old petioles and leaf blades were 60 and 35 per cent. respectively of those in the young parts. The situation in *Rumex* consequently differs from that found in rhubarb in that the acidity of young leaf blades is greater and that of old leaf blades less than that of petioles, while the petioles of rhubarb are always much more acid.

At the close of their resumé of the literature on the origin of organic acids in leaf tissues, VICKERY and PUCHER (**36**) remark that the synthesis and transformation of the organic acids in higher plants are even more obscure than is the case in the molds and yeasts. It may also be said that in no part of the field is the obscurity greater than in that relating to the rôle of oxalic acid in green plants. No generalizations seem possible, since practically every fact established by the study of one plant is definitely contradicted by the findings in regard to another plant, as instanced in the preceding paragraph. In the case of rhubarb, it seems to be clearly established by the work of STEINMANN (**32**) that there is a continuous production of acid, assumed by him to be oxalic acid, in the leaf blade when the leaves are exposed to light; and that translocation of the acid through the veins into the petiole occurs both in light and darkness, so that there is a concentration gradient rising from a minimum at the leaf tip through the veins and midrib to a maximum in the middle of the petiole. That soluble oxalates are transformed into insoluble form in the petiole is evidenced by ANGERHAUSEN'S results. Data here presented show that the maximum concentration of titratable acids found in leaf and petiole throughout life is found in the period of full photosynthetic efficiency, with lower concentrations in juvenile and senescent stages. These facts correlate the rate of production of acids with the general level of photosynthetic activity.

They leave the questions as to the mode of origin and rôle of oxalic acid in the plant unexplained. Whether it is a product of deamination of amino acids, as suggested by RUHLAND and WETZEL, or an intermediate product of photosynthesis playing a rôle in the metabolism of the plant analogous to that of simple sugars in other plants, as suggested by STEINMANN, or a waste product resulting from the operation of an insufficient respiratory mechanism which cannot carry the decomposition further, as the older literature implies, can be determined only by further research.

The course of the changes in nitrogenous constituents in the leaf is rather uniform in the several series. Total nitrogen and amino nitrogen are maximum in the youngest leaves and decrease rather steadily until the leaf is full-grown, after which amino nitrogen becomes fairly constant while total nitrogen undergoes further decrease until the leaf is senescent. There is rather close parallelism between the curves for the decrease in total solids and those for decrease in total nitrogen in the several series of leaf samples (figs. 1 and 4), which suggests that the reduction in amount of total nitrogen on the basis of reduction in percentage of fresh weight is due largely to dilution of the solids with water. In the initial samples of the several series, total nitrogen forms 5.8 to 6 per cent. of the total solids of the leaves; in the final samples it forms 3.15 to 4.5 per cent. of the solids. There is consequently no such large reduction in total nitrogen as SWART (**33**) and TUCKER and TOLLENS (**34**) found to occur in the leaves of *Platanus* and a number of other trees and perennial shrubs. In the case of *Platanus*, this reduction amounted to more than three-fourths of the total. The persistence of a relatively high content of total nitrogen in the old leaves does not disprove but certainly lends no support to the theory of RUHLAND and WETZEL (**27**), who attribute the formation of oxalic acid to the deamination of protein. Moreover, if the results for amino nitrogen are to be relied upon, its uniformly low concentration in petiole and leaf at all times would appear to militate against the possibility that deamination can be occurring at a rate sufficient to account for the large amount of oxalic acid and its salts present in the tissues.

A unique feature of the metabolism of the plant brought out by the present study is its remarkable capacity for absorbing and storing nitrates in amounts very far in excess of any subsequent needs. The work of WOO (**39**), CAMPBELL (**12**), and NIGHTINGALE (**23**) has made it evident that a wide variety of annuals and biennials, in their earlier stages of development, have high nitrate-absorbing capacity and store considerable amounts of nitrates in the aerial parts. Apparently high nitrate absorption is a function definitely limited to the early vegetative stages, since the plant becomes nitrate-free later in the season as flowers and seeds develop, and remains nitrate-free so long as it continues to live. In this respect, as in

some others, the rhubarb plant presents a very different situation. The young and rapidly growing petioles and leaves are low in nitrate content, its utilization in the construction of protein apparently keeping pace with absorption and transport from the roots. But with the completion of growth of the leaf there is rapid increase in nitrate content of both leaf blade and petiole, but more especially in the latter, which continues without material slackening in rate until the leaf blades are yellowing and dying. Series of leaves developed early and late in the season, hence both before and after the usual period of development of the flower stalks, behave alike in this respect. Such differences in amount of nitrates stored as occur in the earlier and later series appear to be attributable to seasonal reduction in the soil nitrate supply and in available water rather than to any alteration in the capacity of the plant for absorbing and storing nitrates. That the nitrates so accumulated are never used in the petioles or translocated elsewhere is conclusively shown by the fact that the petioles of senescent leaves have maximum amounts, the total present in one case when calculated as molecular nitrogen reaching 1.53 per cent. of the dry weight of the plant. Furthermore, the course of the changes in nitrate content of the root system, which increases from April to May and then remains constant throughout the season, shows no relation to the changes in the petioles and leaves. The nitrates of the root are not depleted by the accumulations of nitrate in the petioles of the successive series of leaves, nor is there any indication that they are increased by translocation of nitrates out of the petioles as the leaves complete their life history and die. Lastly, there is obviously no appreciable utilization of the accumulated nitrates in the formation of amino acids or protein in the mature or aging petiole, since its content of total nitrogen decreases progressively from the unfolding of the blade to old age. There was in no case any development of flower stalks, so that we are without knowledge as to what would occur to the nitrates of the petioles in the course of development of flowers and seed.

Summary

1. The biochemical changes occurring in the roots, petioles, and leaf blades of the rhubarb plant in the course of the season's growth were followed by the collection and analysis of four series of samples, each consisting of subsamples of petioles and leaf blades of varying ages from 5 days onward to senescence, at intervals during the season. The first series consisted of the first leaves developed by the plants in spring, which were collected at intervals from April 21 to June 15; the later series was obtained from leaves developing in sequence and were taken on June 5, July 6, and October 17. Samples of the roots were taken on the same dates.

2. The seasonal march of climatological conditions during the period of development and collection of the samples exerted an appreciable influence upon the rate of development of the different series, the petioles and leaf blades of the later series attaining full size in a much shorter time than did those of series I and II. The chief environmental factor to which the more rapid development of the later series is probably attributable is higher mean temperature.

3. The general composition of the several series shows some differences which appear to be attributable to another environmental factor, namely, rainfall. Series I and II developed in a period of ample rainfall very equably distributed over the whole period of growth; series III developed in a period of considerably reduced rainfall; series IV developed in a period of well established drought, the total rainfall for 30 days preceding sampling being 0.55 inch. The reduction in precipitational water supply is reflected in the lower water content of both petioles and leaves at all stages of growth in series III, developed in a period in which transpiration was maximum. The effect of drought during development is also apparent in the leaves of series IV, which retained until senescence set in the high solids content characteristic of developing leaves.

4. Total solids content was maximum in the leaf blade at the time the blades were emerging from the soil and still partially folded, decreased rapidly during growth of the leaf, and became mostly stationary after growth ceased. In the petioles there was a decrease in total solids during rapid elongation, followed by an increase which brought the solids content of the old petioles somewhat above that of the young ones.

5. The alcohol-insoluble solids of the leaf blade decreased progressively throughout the life of the blade; those of the petiole were fairly constant until growth was completed, then increased somewhat in the old petiole. Alcohol-soluble solids of the leaf blade decreased rather steadily throughout life; those of the petiole decreased during rapid growth, then rose as growth was completed.

6. Leaf mesophyll and petiole were practically starch-free at all ages. The sugar content of both was always low, that of the leaf blade being usually highest before the blade had unfolded, decreasing somewhat during growth and fluctuating within narrow limits in the full-grown leaf. Sugar content of the petiole was generally minimum during rapid growth, rising slightly in the old petiole. The fluctuations in sugar concentration in petioles and blades are independent and there was no evidence of the existence of a concentration gradient such as would be expected if large amounts of sugar were transported from the leaf through the petiole.

7. The acid-hydrolyzable polysaccharides of the leaf in two series increased slightly up to attainment of full size; in the other series there was

a slow decrease from youngest to oldest leaves. The acid-hydrolyzable material of the petiole remained stationary or increased within very narrow limits. In both parts its concentration, like that of sugars, was always low.

8. The titratable acidity of the leaf mesophyll and petiole increased during rapid growth, then declined somewhat in the older stages. The acidity of the petioles was at all ages 150 to 250 per cent. that of the leaf blade. In some cases titratable acids, calculated as malic, made up 18 to 25 per cent. of the dry weight of the petiole.

9. The total nitrogen content of leaf blades and petioles declined gradually from youngest to oldest samples in every series. The reduction was relatively much less than that reported for the leaves of woody perennials and trees.

10. Nitrate nitrogen was present in extremely small amounts in the young leaves and increased considerably after growth was completed and the leaves became old. The youngest petioles had seven to eight times as much nitrates as the corresponding leaf blades and increased steadily to a maximum in the senescent petioles, which might have a nitrate nitrogen content equivalent to 1.53 per cent. of the dry weight. There was no indication that this nitrate is used or translocated elsewhere.

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MECHANISM OF THE ACTION OF LIGHT AND OTHER FACTORS ON STOMATAL MOVEMENT

GEORGE W. SCARTH

Introduction

The factors which have been reported to influence stomatal movement are: (1) *light*; (2) *turgidity* of the leaf; (3) *temperature*; (4) physiological *shock*; and (5), under artificial conditions, certain *chemical agents*. On the efficacy of light and water supply there is general agreement, but the conditions of their respective dominance are not well understood and the mechanism of their action is still the subject of diverse theories. As regards temperature and shock, investigators differ on the question of whether these factors have any important effect at all on the stomata of plants in general.

With the possibility of all these agents interacting and interfering with one another in the experiments, it seemed desirable to make a broad survey of the action of all of them on the species worked with before prosecuting more intensive study of any one. The results of this preliminary survey are given in the present paper, as well as some more detailed work on the action of light. Further investigation on the effect of temperature is reported in a paper which will follow. Although these experiments have been chiefly confined to one species, *Zebrina pendula* (*Tradescantia zebrina*), occasional observation of other species has yielded no evidence that the physiology of *Zebrina* is in any fundamental way unique, although, of course, plants differ in their relative sensitivity to the respective factors. Since the object of the research was not to obtain quantitative data regarding the responses of the stomata of a particular species to environmental conditions but to investigate the mechanism of that response, which, like other deep-seated cellular processes, is probably little subject to specific differences, it seemed unnecessary to particularize in the title of this paper as to the species studied.

The changes in the stomatal apparatus which were principally followed may be enumerated at this stage, and some of their known relations pointed out.

The function of a stoma as a ventilatory organ is determined by the linear dimensions of its aperture. The *width of the slit*, therefore, was taken as the basic character with which the other variables were to be correlated. This was generally measured *in situ*, but sometimes after fixation in alcohol, after the method of LLOYD (**16**). The width of the opening depends upon the *shape* of the guard cells, which changes with their own vol-

ume and that of the adjoining cells. The *diameter of the whole stoma* was accordingly measured in some cases, as a further index of these morphological changes. The immediate dynamic causes of alterations in the shape and size of the guard cells are changes in certain turgor pressures: first and foremost is that of the guard cells themselves (because, as several workers have shown, this changes to a much greater degree than the following): secondly, is that of the subsidiary cells and, in diminishing degree, of more remote cells. As to the rôle of the neighboring cells, the shape of the guard cells is modified considerably by the mechanical pressure of the flanking cells but, on account of longitudinal ridges in their own walls, little if any by those cells which abut on their ends. We may say, therefore, with URSPRUNG and BLUM, that it is the difference between the turgor pressure of the guard cells and of these subsidiary cells which determines the stomatal aperture. At equilibrium these turgor pressures vary as the osmotic pressure or "osmotic value" of the respective cells. It was enough for the purpose of this study to take the *osmotic value at incipient plasmolysis* as a standard of comparison. This was done frequently for guard cells and sometimes for the subsidiary cells also, using $CaCl_2$ or cane sugar solutions. When equilibrium does not exist, that is, when the suction pressure of the guard cells and subsidiary cells respectively is not equal, their turgor pressures are determined in part also by the external forces which bring about this inequality. Owing to their position and the cuticularization of their free walls, the guard cells seem both to gain and to lose water by way of the subsidiary cells. At any rate, a hypertonic solution applied to a section withdraws water from and reduces turgor in the subsidiary cells more rapidly than in the guard cells, causing temporary opening of stomata. The same has been reported frequently as the initial effect of wilting. Similarly water, whether applied to a section or injected into a leaf, tends at first to promote closure. For this and other reasons, in all the experiments except those specifically directed to test the effect of wilting, the leaves have been kept turgid.

Effects of reduced turgor will be further described in the section devoted to that topic.

Going a step farther back to the physico-chemical mechanisms of the osmotic changes, it is well known that in the guard cells reversible transformation of carbohydrate from sugar to starch and back again is of major importance, at least in the diurnal cycle; but nothing is definitely known as to whether addition and removal of carbohydrate may also play a part. If and when this happens, we should expect to find that the osmotic value and the amount of starch do not vary reciprocally. *Changes in the starch content* of the guard cells were therefore estimated in most of the work.

This was done by comparing the size of the starch grains after decolorizing with alcohol and staining with iodine in KI.

In a former paper (**22**), some evidence was presented of turgor being determined to some extent by other and more rapid processes, including, it was suggested, changes in the hydration capacity of colloidal material in the cell sap. This aspect of the problem was not pursued in the present study because, in the course of any major movement, such change, if it is a fact, is always accompanied or followed by the more easily observed hydrolysis or synthesis of starch, and my principal object was the investigation of the more fundamental processes through which both the osmotic and colloidal changes are linked with the external factors that govern them.

In the same paper (**22**) it was stated that guard cells stain differently with certain indicators, according as the stomata are open (in light) or closed (in darkness). The results point to an increase of pH in light. On account of the many difficulties and sources of error attending this kind of work, these experiments were repeated and extended. In addition, similar tests were applied in relation to the action of other factors than light. The technique of these indicator tests will be described in the section on the effects of light.

The various factors are considered separately, in the following order: (1) shock and wound stimulus; (2) temperature; (3) turgidity of the leaves; (4) light; (5) chemical agents which affect the pH of the guard cells.

I. Effects of shock and wound stimulus

Before we can satisfactorily study the action of environmental factors on stomatal movement, it is necessary to take stock of any possible effect of the mere manipulation of the material in the process of experimentation. Mechanical stimulation of the plant or leaves by shaking, rubbing, exposing to blasts of air, etc., which was found by KNIGHT (**10**) to induce partial and temporary closure in certain plants, seems to have no effect on *Zebrina pendula* or (so far as observed) on any other species employed in the present work. Sudden pronounced changes of temperature produced slight temporary movements, as will be described later, which may fall into the category of "shock" effects. More important movements are produced by actual injury of a portion of the leaf. If, for example, by touching with a hot glass rod, by applying a drop of strong acid, or by cutting with a razor, some of the cells are killed, stomata in the immediate vicinity of these cells tend to close. No effect is noticeable more than a millimeter or so away from the actual wound; but since sectioning is so commonly used to expose cells for observation and treatment, it is very important to discover what changes in their properties are produced by the operation, and to distin-

guish between the effect of the stimulus transmitted from the wounded cells and the change of physical environment to which cells in the neighborhood of a cut are subjected.

EXPERIMENTAL RESULTS.—Sections from the lower surface of a leaf of *Zebrina,* and also whole leaves of which the uncuticularized cell walls of portions of the lower epidermis were exposed by shaving off the overlying tissue, were placed in various environments and the behavior of stomata thus "exposed" was compared with that of normally "covered" stomata. The experiment was usually begun with open stomata and the preparations were kept in the light, so that the "covered" stomata remained practically unchanged or, if the experiment was prolonged overnight, they underwent normal changes in illumination. When the preparation is immersed in water, the "exposed" stomata close rapidly in spite of continued illumination. Visible closure begins within a few minutes and may be complete in some of the stomata within 15 minutes—a much shorter period than is required for ordinary closure induced by darkness or wilting. This applies to stomata with intact subsidiary cells. If the latter are destroyed by the cutting, so that their pressure on the guard cells is removed, the stomata immediately widen and do not close later; but the same internal changes take place in these guard cells as in those of intact stomata. As regards the latter, the rate and degree of closure decrease with their distance from the cut surface, and movement is not appreciable beyond the thickness of a few cells.

In water freed of CO_2 but otherwise aerated, the early behavior is exactly the same as in tap water. The closure, therefore, is not due to the slight increase in the CO_2 concentration of the environment.

In moist air or in oil (nujol) the exposed stomata still close, but less rapidly, and, on the whole, to a less degree. Evidently the presence of free water, temporarily at least, promotes closure. This effect of water is also seen when uncut leaves are infiltrated with water. Stomata over water-filled stomatal chambers do not, as a rule, open quite so widely as over air-filled chambers. No doubt the subsidiary cells and other parts of the epidermis are usually unsaturated and become more turgid in presence of water; while, relatively, the guard cells either take up water more slowly or in less degree.

The fact that even in oil or air the stomata near a cut surface quickly close, however, indicates that changes in tissue tension are not the sole or main cause of the movement. This is proved more directly by a study of the osmotic properties of the guard cells. The osmotic value at incipient plasmolysis was observed to fall markedly as closure proceeds. In one case, for example, the guard cells of "covered" stomata were plasmolyzed in $M \div 3$ but not in $M \div 4$ $CaCl_2$, whereas in the same preparation "exposed"

guard cells half an hour after cutting were mostly hypotonic to M ÷ 6 $CaCl_2$. Little increase in starch can be observed while the osmotic value is falling, at least for half an hour or so. It is possible, of course, that the sugar which is abundant in open stomata is converted into some osmotically inactive carbohydrate other than starch, but the speed with which the osmotic pressure is reduced and the persistence of low pressure long after the actual physiological shock effect should have worn off point rather to a temporary increase of permeability and loss of solute from the guard cells than to a reversible condensation of carbohydrate within them. On the day after cutting, the "exposed" guard cells still fail to open in light and their osmotic value remains low. Sometimes also their starch content is now high, compared with that of "covered" guard cells in the same preparation. This starch increase was not observed in water free of CO_2 and may be the result of pH difference, as explained later.

From these experiments it is obvious that we cannot arrive at a knowledge of the behavior of stomata under normal conditions from a study simply of those near a cut surface. Fortunately, however, the reagents usually penetrate beyond the range of the more serious effects of the injury and they can also be introduced into whole leaves without having resource to cutting. These precautions are particularly necessary in staining with indicators. As regards the solutions ($CaCl_2$ and cane sugar) used in estimating osmotic value, it would seem that, if applied immediately after cutting, they counteract in large measure the injury effect, presumably inhibiting the increase of permeability; otherwise there would not be such high osmotic values in the guard cells of open stomata. These high values are evidently not due to penetration of the reagents, because there is no subsequent deplasmolysis in either $CaCl_2$ or cane sugar. On the other hand, there *is* a general fall in the osmotic value of guard cells initially open, when these remain for hours in the solutions mentioned; but this is accompanied by increase of starch and is probably due to condensation rather than to exosmosis of sugars.

No pronounced change in the osmotic value of the epidermal cells was observed to result from sectioning. It would seem, therefore, that the fall in turgor as a response to injury of neighboring tissue is more or less specific to guard cells and particularly to those of open stomata.

In a paper published several years ago, AREND[1] has recorded the rapid closing of stomata of *Zebrina* in the vicinity of a wound. In one important point, however, my observations fail to agree with AREND' and repetition of the experiment does not alter my opinion. AREND says that starch is rapidly formed in the guard cells as the stomata close, reaching its maxi-

[1] AREND, J. Ueber den Einfluss chemischer Agenzien auf Stärkegehalt und osmotischen Wert der Spaltöffnungschliesszellen. Planta 1: 84–115. 1925.

mum in 20 minutes, after which time no further condensation can be observed. I find, on the other hand, that if starch is absent to begin with, then whether in light or darkness, no more than mere traces can be detected after 20 minutes, even in the guard cells of stomata which have closed completely; moreover the starch gradually increases in amount for several hours after wounding. When the wound response was first observed, I was particularly watchful for starch production, because the hypothesis was entertained that closure might be caused by "acid of injury" developing in the guard cells or neighbouring cells, the production of which in guard cells near a wound is suggested by the reaction with indicators, as described later. But whereas the rate of starch condensation after wounding is of the same order as in intact leaves with open stomata when subjected to acetic acid vapor, the closing movement is much more rapid than when produced by acid, darkness, or wilting; and complete closure is attained with much less (if any) starch formation than under these other conditions.

The possibility of temporary accumulation of some other osmotically inactive carbohydrate than starch has already been mentioned, as well as my reasons for thinking that this is not sufficient explanation of the anomaly, and that escape of solute is at least a part of the mechanism of the drop in turgor.

It may be added that closure of stomata near a wound has been observed in many species of plants besides *Zebrina*.

II. Effects of temperature: preliminary experiments

Many investigators have found that stomata tend to open with increase of temperature; many others that they do not; and others again that the behavior is variable. In a few preliminary experiments with detached leaves of *Zebrina* kept in water, the writer found that high temperatures (38° to 40° C.) not only accentuate stomatal opening in light but tend to cause it or prevent closure, as the case may be, in darkness; while low temperatures (0° to 8° C.) prevent opening in light. Moreover, when some of the guard cells in such leaves are stained with methyl or ethyl red, as described in the next section, the quality of the color varies in such a way as to indicate that the pH of the cell sap increases with rise of temperature.

These experiments showed the necessity of temperature control in testing the effects of other factors. Accordingly, in the experiments which follow on the action of light, this control was practised whenever the light was sufficiently intense to make it necessary.

More systematic study of the effects of temperature on the properties of the guard cells was carried on with the help of a colleague and will be reported in a paper which will follow. This later work confirms the pH

change and shows that it is accompanied by the same transformation of carbohydrate as in the response to light.

III. Effects of leaf turgor

The relation of stomatal opening to the amount of water in the leaf is a very complex one. Whether turgidity be above or below normal, the result varies with the duration of the abnormal state. It also varies considerably with the species of plant and the age and position of the leaves, while, of course, it is modified by the other external factors which regulate opening (BURGERSTEIN 1).

As regards the time factor, three phases have been distinguished in the process of wilting. (1) There is considerable evidence of an opening movement in the early stages, in certain plants at least—although this has been observed directly in only a few cases, *e.g.*, by STALFELT (**23**) and SAYRE (**20**). The initial increase of transpiration observed by DARWIN (**2**); and even the rise in porometer readings of LAIDLAW and KNIGHT (**14**) may conceivably be due to non-stomatal regulation, as argued by LLOYD, who was unable to observe any initial opening of stomata during wilting. Apparently plants differ in this respect. (2) More pronounced wilting results in closure in the majority of plants. According to various workers, however, a considerable percentage show open stomata. BURGERSTEIN, who investigated a great number of species in this regard, found that 16 per cent. of the herbaceous plants and 10 per cent. of the woody plants examined showed wide open stomata in the wilted state; while 40 per cent. herbaceous and 23 per cent. woody plants showed only partial closure. Such data, however, ought to be considered in relation to light and temperature as well as to water supply. The reduction of transpiration which automatically accompanies wilting may result in a rise in temperature of the leaves, if they are exposed to direct insolation. (3) In an extremely dry and, as BURGERSTEIN believes, posthumous state of the leaves, stomata which were previously closed may open.

The early opening is probably a direct effect of the reduction in the amount of water in and pressure of the neighboring cells; the secondary closure is certainly an indirect effect which consists, as shown by ILJIN (**6**) and STEINBERGER (**25**), in a reduction of the osmotic pressure of the guard cells through transformation of sugar to starch, just as in darkness; the final opening, when it occurs, may again be a direct action, namely, the result of tissue shrinkage.

Usually high turgidity, like low, brings a chain of changes rather than a single response. Infiltration of the leaves of *Zebrina* with water tends to reduce the stomatal aperture slightly. This is a direct and almost immediate effect, but a high state of turgor also has a less immediate indirect

effect. It favors starch hydrolysis in the guard cells and stomatal opening in light, as has frequently been observed, and also induces it in darkness, as the writer's next paper will show.

A few personal observations may be added to the preceding statement of the effects of wilting. In the first place, it is very noticeable that stomata and even guard cells respond individually and not *en masse*. Some stomata may become completely shut before others adjacent to them are scarcely contracted, and the inner edge of one guard cell of a pair may be straight while the other is still half bent. In the second place, *the guard cells of wilted leaves exposed to the light stain with methyl and ethyl red like those of leaves kept in the dark and not like those of turgid leaves in the light;* that is to say, they stain pink with a dye solution which appears practically colorless in illuminated turgid leaves. This result was obtained when sections from the respective leaves were placed for a short time in the stains, but a similar result was obtained when leaves infiltrated with and partially dehydrated by a sugar solution containing dye were compared with leaves infiltrated with an aqueous dye solution after both had been exposed for some time to light. This staining distinction points to a difference in pH of the cell sap in turgid and wilted leaves in the light. In later experiments, to be reported in the next paper, a similar distinction was noted in the case of leaves kept in the dark. The principal point to be noted is that the major movements of stomata in response to changes of leaf turgor are accompanied (1) by starch-sugar transformation in the guard cells (as is already known) and (2) by staining differences which indicate a change of pH.

What significance these observations may have on the mechanism by which the water relations of the leaf regulate the osmotic value of the guard cells can be discussed more conveniently in the light of experiments which follow.

IV. Effects of light

A picture of the series of events which takes place in the guard cells under the influence of light was adumbrated in the work of DARWIN (**2**) and ROSING (**19**), and more clearly delineated by LLOYD (**16**), ILJIN (**6**), HAGEN (**5**), and others. They are chiefly disappearance of starch and increase of sugar from which ensues the osmotic intake of water that causes opening. Other light effects, first noted by WEBER, are increased resistance to heat, decreased adhesion of the protoplast to the wall, cessation of plasma streaming, a more scattered distribution of the chloroplasts, and changes in the form and visibility of the nucleus and nucleolus. These changes are probably incidental and not directly concerned with the opening mechanism, but any theory of that mechanism should account for them also.

KISSELEW (9) and NIKOLIC (18) argue that the speed with which stomata sometimes change their posture, especially in the direction of closure, points to a change of permeability rather than osmotic pressure. My results, already described, on the effect of treatments which may be regarded as causing "shock," agree with this; but the ordinary photic response (or by far the greater part of it at least) is gradual and proceeds *pari passu* with change in the osmotic value of the guard cells not to be accounted for in terms of permeability.

Perhaps this statement may require modification if the progress of movement is minutely analyzed. Fluctuations in the earlier stages of opening have been recorded by NIKOLIC (18) and STALFELT (23). On the basis of relation to the light stimulus, STALFELT distinguishes the "stretching phase" of opening up to the first appearance of an aperture and the "motor phase" from there onward. The progress of the second depends quantitatively on the amount of light received, *i.e.*, on the intensity × time (beyond a certain minimum intensity), while that of the first stage does not so depend. It is also fluctuating, but so (according to STALFELT) is the movement in darkness in leaves and shoots which have been removed from the plant. In view of the rather complex adjustments of suction tension and turgor between the guard cells and subsidiary cells, which follow any change in the relative rate of gain and loss of water by the leaves, the apparent periodicity of this response does not necessarily imply that it fails to fit into any of the categories which we are considering. At all events, the present study deals with only the major trends of movement and with the links between these and the environmental changes to which they are due.

Another effect of light on the guard cells, which was previously reported by the writer and for which the evidence is now fully presented in this paper, is a modification of their staining with certain indicators, pointing to an increase in the vacuolar pH. That this change may be essential to the mechanism rather than merely incidental was suggested by the similarity of the results produced through artificially varying the acidity of the guard cells to those produced normally by changes in illumination. In an earlier paper (22) it was shown that when epidermal sections of a leaf are kept for some time in a dilute solution of ammonia, even in the dark, starch disappears from the guard cells and the stomata tend to open, while dilute acetic acid, even in the light, produces the opposite effect. Moreover, the subsidiary changes just mentioned were in large measure duplicated under this treatment. On the basis of these results, the writer went so far as to put forward the working hypothesis that the ordinary effects of light and darkness respectively act through the intermedium of a pH change, as determined probably by the relative rate of assimilation and production of CO_2 —and possibly other acid products of respiration. To test this hypothesis,

further experiments have been carried out on the relation of stomatal behavior to light and to hydrogen-ion changes.

The principal points of the various experiments will be clearer if they are grouped according to the problem toward the solution of which they were designed to contribute.

(1) *Are the changes in staining property of the guard cells which are induced by changes in illumination really indicative of a shift in the pH of their cell sap?* Under strictly controlled conditions, in which the leaves and indicator solutions and all environmental factors, except light, are invariable, the guard cells of *Zebrina pendula* (and other plants tested), if they stain at all with methyl red, ethyl red, or propyl red, always show differences of staining in relation to whether the leaves have been exposed to light or darkness. Sometimes this difference amounts only to a more rapid absorption of dye in leaves taken from the dark and deeper staining in a given time or to uncertain differences in tint; but often the color distinction is qualitative and clear cut, guard cells from the dark appearing pink and from the light colorless. The latter immediately become equally pink when treated with acetic acid, which proves that in lighted guard cells the indicator is present but on the alkaline side of the point of virage.

This color difference in the staining of the guard cells can be observed in sections which are quickly stained and examined after being taken from the variously treated leaves. They can also be seen in thick sections which are first stained and then exposed to light or dark respectively. They are more pronounced in leaves which have been partly infiltrated with dye solution, either before or after the treatment as regards light. The distinction is not dependent upon whether the stomata are closed or open because, for example, on exposure of leaves to light the indicator reaction begins to change *before* the stomata open or when only a slight slit is showing.

Having observed these differences hundreds of times, the writer is convinced that they are not accidental, although the result may easily be upset by a difference in the type of leaf or in the reaction of the indicator solutions, as already pointed out. The simplest explanation of transformation of the indicator from red to yellow or yellow to red is a change in H-ion concentration of the cell sap. Even if the property of binding the stain in its red state is imparted by the production of a negatively charged colloid, such a colloid is likely to be an acidoid, *i.e.,* one which dissociates H-ions into the liquid phase.

(2) *What appears to be the range of the pH of the guard cells and other tissues as affected by light and darkness respectively?* Whole plants or detached leaves placed in water were kept in the dark for several hours and respectively exposed to the light until their stomata were well open. The leaves were then infiltrated with various indicators, and after 10 minutes

or so they were washed and the under surface and sections from it observed under the microscope. The color of the indicator in the intercellular spaces underlying the stomata was noted in the intact leaf and the color in the guard cells observed either in the intact leaf or in sections. Guard cells and intercellular spaces vary together. The most acid reaction observed in the guard cells was red with methyl red, but yellow with benzene red (*i.e.*, apparently between pH 4.8 and 5.0). The most alkaline reaction was purple with brom cresol purple (the only acidic indicator that could be detected in the guard cells), but red with neutral red (*i.e.*, apparently between pH 6.0 and 7.0).

There is possibility of considerable error in the turning point of neutral red, which is very strongly absorbed by the cells and does not change to yellow very readily when weak ammonia is applied. The fact, however, that the flavone pigment naturally present in guard cells, as well as in the other epidermal cells, does not become yellow in the light indicates that the pH does not extend at any rate beyond pH 7.4, up to which point the flavone is colorless. (The turning point of the flavone was determined by extracting the mixture of anthocyanin and flavone pigments which are present in the epidermis of *Zebrina* and finding the pH points at which the mixed solution underwent color changes. The solution is reddish-purple (as in the leaf) at pH 7.0, changes to bluish at about pH 7.2 and to green at about pH 7.4 to 7.5. The green is due to the addition of the yellow of the flavone to the blue of the anthocyanin, so that up to about 7.4 the flavone evidently remains colorless.)

Unfortunately the writer has no basic nor amphoteric indicator with range between pH 6.0 and 7.0 to discover more readily the most alkaline limit of the reaction attained by the guard cells of *Zebrina* and *Tradescantia*. However, in *Pelargonium* leaves exposed to sunlight, neutral red in the guard cells sometimes has a brick red or orange hue, differing from its pink shade in the other cells and indicative of an almost neutral pH.

As regards the pH range of the liquid in the infiltrated leaf spaces, the most acid reaction—observed once or twice in *Zebrina*—was greenish with brom cresol green (turning blue with ammonia) and reddish with methyl red; *i.e.*, around pH 4.0. The most alkaline reaction was blue with brom thymol blue; *i.e.*, over pH 6.6. Brom cresol purple has a useful range (pH 5.8 to 6.2) for qualitative demonstration of pH change in the leaf spaces. The color of this indicator in the stomatal chambers can be changed to and fro from purple to yellow, according as the infiltrated leaves (lying in a water bath) are kept in light or darkness. (The yellow, as with all these dyes, is practically invisible as observed under these conditions.)

What partial pressure of CO_2 is necessary to change the particular dye solution used to the most acid reaction observed in the intercellular spaces,

a pH of 4.2 or less, was not determined. With distilled water, 20 per cent. CO_2 would be sufficient to give a pH of about 4.0, but in the solution the buffer action of the dye would require a higher concentration of CO_2 to produce the same effect. Twenty per cent. CO_2 has been measured in potatoes. In potato sap 20 per cent. CO_2 produces a pH of only about 6.0; but guard cells, in view of their natural fluctuations of pH, are probably much less buffered than potato cells.

As regards the cells of the leaf other than the guard cells, no definite results were obtained as to their pH or changes therein. The indications are that it is fairly constant. Acidic indicators, in particular brom cresol purple, were allowed to accumulate in the leaf cells of *Anthericum* by setting cut leaves in a solution of dye, but no appreciable difference in coloration was noted according as the leaves were kept in light or darkness.

To summarize, the effect of light *vs.* darkness on the indicator reactions in the leaves is as follows: (a) In the intercellular spaces, *i.e.*, in a concentrated solution of the indicators in equilibrium with the internal atmosphere of the leaf, there is an apparent range from over pH 6.6 in light to about pH 4.0 in darkness. (b) In the leaf cells in general there is no great change so far as could be determined from this incomplete study. (c) In the guard cells there is a wide change from between pH 6.0 and 7.4 in light to less than pH 5.0 in the dark.

These results demonstrate a correlation between the concentration of CO_2 in the leaf spaces, the pH of the guard cells, and the osmotic changes within them. So far as I have gone at present, however, they do not prove a causal relation between these phenomena. The photosynthetic action of light and its stimulation of the guard cells may be entirely independent. The relation between these two actions is the next subject of inquiry.

(3) *What relation does the effect of light on stomata bear to its photosynthetic activity?* Two lines of investigation were followed: (a) the effect of light with respect to its wave-length and its absorption by chlorophyll; and (b) the effect of light on stomata separated from the green tissue of the leaf.

a. *Effect of light with respect to wave-length:* Kohl (**11**) found that of the visible spectrum only the region between the *B* and *C* lines, that is the red, and between *F* and the violet, that is the blue, were effective. Lloyd (**16**) found the blue to be less effective than the red. Kohl showed that infra-red radiation also causes opening, but, as later experiments will show, this may be a temperature effect.

In the experiments which follow, regarding the action of light of different wave-lengths on the pH as well as the posture of the guard cells, temperature has been kept constant. Dishes containing detached leaves were set in running water and with a water screen between them and the

source of light—a 150-watt Mazda lamp placed about 6 inches above the preparation. The dishes, each containing two leaves, were covered respectively by the following filters: a ground glass screen, an opaque screen, and two Wratten and Wainwright filters, allowing only red, green, and blue light to pass. The leaves, taken from plants which had been kept in the dark overnight or longer, were slit in numerous places and centrifuged for about two minutes in saturated ethyl red; about 10 minutes later, by which time guard cells near the slits were stained pink, the leaves, still lying in dye solution, were exposed as described to various types of illumination.

After an hour or more the stomata always opened somewhat (slightly to half open) under both white and red screens, and the stain in the guard cells faded greatly or sometimes disappeared; *i.e.*, became yellow. In darkness the stomata remained shut and the stain intensified. Under the green and blue screens the result was sometimes indistinguishable from that of complete darkness, although at other times there was slight opening and reduction of stain. To obtain pure blue light, two Wratten and Wainwright screens had to be employed and the intensity of the light transmitted as very small. There was more evidence of activity with the Corning blue glass screen no. 10. The intensity as well as the quality of the transmitted light would have to be regulated in order that the relative efficiency of the spectral regions might be determined, but this analytical mode of investigation was not pursued very far because it seemed possible to arrive at the information required more simply and directly by using chlorophyll itself as the color screen. It also seemed better to use rooted plants than detached leaves, since fuller and more regular opening of the stomata takes place in the former.

Potted plants well supplied with water were placed in earthenware crocks and covered by dishes containing liquids with different light-absorbing qualities: (1) India ink; (2) chlorophyll in alcoholic solution; (3) chlorophyll with Cu substituted for Mg; (4) solutions of red dyes, such as basic fuchsin and safranin, which transmit the red band absorbed by chlorophyll. The chlorophyll was changed every half-hour or hour, or at longer intervals (depending on light intensity) before any sign of decomposition or colloidal precipitation appeared. The preparations were simultaneously exposed either to diffuse daylight or in some cases to direct sunlight. It was found that a clear transparent chlorophyll solution may completely inhibit the light activity under conditions of illumination in which an India ink solution two or three times as opaque to the eye and basic fuchsin or safranin of equal opacity allow full and normally rapid opening. (Stomata of *Zebrina* open well in very weak daylight.)

Although the only measure of intensity used was brightness to the eye, the difference between the effect of chlorophyll and other solutions is so great that there can be no doubt that it is the quality, not the intensity, of the light transmitted through chlorophyll that is effective in producing opening of stomata. In other words, only the wave-lengths most strongly absorbed by chlorophyll are appreciably active in causing opening of stomata in the green leaf.

b. *Effect of light on stomata separate from chlorenchyma:* To avoid the wound response, these experiments were performed on variegated leaves, chiefly of *Pelargonium, Abutilon, Pandanus veitchii,* and a species of grass. The object was to determine whether the guard cells or the chlorenchyma or both absorb the light which causes opening.

KÜMMLER (**12**), using these and other variegated species, found only minimal opening in white parts with medium humidity and normal light and temperature, although in air saturated with moisture he found that light might induce maximal opening in some of the stomata of white areas. VELSEN (**26**), using the porometer method, found much less opening in parts of *Ficus* leaves which lacked green tissue.

In all these plants there is some pigment in the plastids of the guard cells. In *Abutilon* it is distinctly green; in *Pandanus* a very pale green; and in white-edged *Pelargonium,* according to my observation, very pale yellow or greenish yellow and hard to perceive. KÜMMLER describes the guard cells of this form as "chlorophyll free," but there seems to be some pigment present. Whether the pigmentation is distinct or indistinct, it is in each case equal in the green and white leaves or parts of a leaf. There seemed to be no correlation between pigmentation of the guard cells and reactivity to light.

With white or red light, the *predominating influence of green mesophyll* is conspicuous. In turgid leaves on the plant and leaves lying in water when exposed to light which causes the stomata of green parts to become wide open, little effect may be produced on the white parts. The stomata remain closed or open slightly, except that *near the boundary of the green, the stomata on the white areas open well and the influence spreads, as exposure to light continues.* Reduced amount of chlorophyll is accompanied by reduced opening, as was already noted by KÜMMLER. In the pale, but not white, central portion of a banded *Geranium* leaf the stomata were found to be half open when in the green margin they were fully open with circular shape.

Light which has been filtered by a chlorophyll solution has as previously indicated, no more effect on the stomata of white leaves than of green. As the chlorophyll plus xanthophyll absorbs the shorter wave-lengths, and as this end of the spectrum is most chemically and physiologically active, the

effect of blue-violet light was studied with aid of a screen. Light from a 150 watt lamp at 6 inches' distance, after passing through a water screen and through a Corning glass filter no. 10 (which allows only blue and violet to pass), has scarcely any effect on stomata either in the pale or white portions of the leaves. Sunlight similarly filtered had a slight effect on both, although how much of this was due to rise of temperature is uncertain. At any rate the effect is small, compared with that of red light on green tissue.

A possibility which has to be considered is that light may stimulate the guard cells directly but does so in full measure only when by its action on the chlorenchyma it has produced a favorable environment. The fact that red light alone produces opening just as well as white points to a pure photosynthetic action rather than to a combination of this and protoplasmic stimulation. Also STALFELT's finding that response varies as light intensity × time of exposure suggests that light supplies the energy and not merely the stimulus. The inference is that in so far as light acts directly on the guard cells, it is through the activity of their chlorophyll. At any rate, it is clear from these experiments that stomata separated from assimilating tissue are only slightly affected by the action of visible light of any wavelength.

The fact that some influence may spread from the green tissue for a certain distance into a white area is in harmony with my theory that reduction of CO_2 concentration in the tissue is a fundamental part of the mechanism of light action; but it does not amount to proof of this mechanism because the result may be due to diffusion of some other substance or the transmission of a physiological stimulus, as in the wounding effect.

The feasibility of the former view is supported more directly by experiments on the effect of varying the concentration of CO_2 and the pH of the guard cells in other ways, with or without exposing the leaves to light.

V. Effects of chemical agents (including CO_2) which change the pH of the guard cells

Preliminary to a study of the rôle of CO_2, inquiry was made as to whether the pH change in the guard cells induced by light is merely accidental or is a part of the mechanism of opening. The answer to this question will depend largely upon whether an artificial variation of pH (especially within the range which is induced by light changes) can produce the full effect of light on the guard cells.

The writer's earlier experiments on this subject have already been referred to. In ammonia solutions and in darkness, starch disappears from the guard cells at a rate which increases with the concentration of alkali up to the limits of toxicity. In the higher concentrations the rate of

disappearance is considerably faster than in light. In acid up to a certain concentration, starch is formed in these cells even in the light; but within a narrow range of acidity beyond this and short of a concentration that is rapidly toxic, starch disappears. Prolonged exposure, even to this concentration of acid, results in death of the cells. Accompanying these starch variations reciprocal changes in osmotic value take place with corresponding movements of the guard cells. Under the conditions described, although the starch content may vary from zero to the maximum, the range of movement is small compared with what is found under ordinary conditions in intact leaves. At first sight, therefore, it might seem that the response to these chemical agents is not comparable with the response to light. On the other hand, in sections the wound stimulus and the effect of the aqueous medium tend to prevent opening. In fact, the stomata near a cut surface open to a less extent under the action of light than of ammonia.

Further experiments were therefore carried out, using cut shoots and rooted plants and exposing them to ammonia and acetic acid in vapor form. SAYRE (**20**) has already reported that the stomata of *Rumex patientia* open to 50 per cent. of their maximum in ammonia vapor in darkness and close in an acid atmosphere in light.

In the present experiments the plants were kept in a moist atmosphere under a bell jar, so that the leaves were turgid. The pressure of the reagents in the atmosphere of the bell jar was not measured, but in the case of ammonia, at least, the leaf pigments serve as natural indicators of the concentration of OH-ions in the cells. This is much more significant, because the concentrations of the ammonia or acid vapor outside and inside a leaf do not necessarily vary together. Ammonia strong enough to cause quick color change in leaves with open stomata may have no effect, even after many hours' exposure, on leaves with stomata completely shut.

It was found that under the conditions now described the stomata of *Zebrina* may open almost as widely in ammonia in the dark as under the action of light. As to the intracellular pH at which this occurs, in most experiments the stomata opened only when the purple epidermal cells had changed to green and the guard and subsidiary cells to yellow; that is, at a pH of about 7.4. This condition is usually attended by the formation of a precipitate in the sap of the subsidiary cells, but there is no fall in their osmotic value at this stage (if anything, a slight rise), so that the wide opening is not due to reduction of the pressure of these cells, as might be the case when the ammonia is toxic.

There is evidence, however, that stomata open below pH 7.4. In one case the stomata were found to be wide open with starch reduced almost to nil when the flavone pigment had not become yellow and the anthocyanin had merely changed to a bluish purple shade. Moreover if, as usually

happens when the stomata are initially shut, the color change starts from local points of entry of the ammonia and extends as green patches with a blue margin, the stomata in this blue transitional zone, in which there is no yellowing of the guard cells or any other cells, begin to open somewhat rapidly, although the accumulation of ammonia produces further color change before the opening is complete.

It is inferred from these results that if the ammonia concentration could be controlled, full opening would take place when the pH of the guard cells is just short of the point at which the flavone becomes yellow, about pH 7.4.

The indicator tests reported earlier show that in light the pH of the guard cells may rise at any rate to between pH 6 and pH 7, but do not warrant stating whether it passes pH 7 or not.

The conclusion reached is that light causes the guard cells to approach and, in absence of contrary evidence, possibly to reach a pH which in absence of light will induce almost as wide opening as takes place in presence of light. The inference that light acts through changing the pH is rendered more probable by the fact that, as already reported, numerous intracellular changes in the guard cells are the same in natural and artificial opening. Some of these may be accounted for by the change in shape and pressure of the vacuole(s); others by changes in the physical properties (surface tension, viscosity, etc.) of the protoplasm determined by H-ion concentration.

ACTION OF ACETIC ACID VAPOR.—It was found that the action of acid vapor, if not too concentrated, inhibits opening. Opening was sometimes observed in higher concentration but this was not studied, since the relation to carbonic acid seemed to call for more particular attention.

ACTION OF CARBON DIOXIDE.—When the work of DARWIN (**2**), LLOYD (**16**), and LINSBAUER (**15**) made it clear that light acts equally well in absence of CO_2 (one of the raw materials of photosynthesis), and that, according to LINSBAUER, absence of CO_2 is even favorable to opening, including such as may take place in the dark, the old view that the turgor of the guard cells increased through accumulation of the direct products of photosynthesis had to be abandoned. These facts, however, did not upset the general conclusion to which we have been approaching: that photosynthesis is the active factor in stomatal opening, for even in absence of atmospheric CO_2 the photosynthetic process is still carried on in as far as CO_2 is liberated by respiration. Consequently, STAHL (**24**) put forward the view that light acts through removal of CO_2 and darkness through accumulation of the gas as a product of respiration. This conclusion was based especially on LINSBAUER'S experiments, in which he found not only that a decreased amount of CO_2 favors opening but that an increased

amount causes shutting both in light and in darkness. According to LINSBAUER, this is true of high partial pressures of CO_2 and of the pure gas; but KOSAROFF (**13**) found that the reduction of transpiration in high concentrations of CO_2 was the result rather of lack of oxygen than of abundance of CO_2. By mixing oxygen with the CO_2 the depression of transpiration was reduced as the oxygen was increased.

It seems necessary that the relation to CO_2 and oxygen should be further investigated, especially in the light of the following experiments.

Pot plants, cuttings (rooted and unrooted) set in water, and cuttings not in water, were exposed to various concentrations of CO_2 in a dark moist chamber. In the case of the controls, which were kept under similar conditions but in ordinary atmosphere as regards CO_2, there was a certain amount of stomatal opening in the pot plants after many hours but little or none in the cuttings.

In CO_2 of 15 per cent. concentration and upward, the stomata began to open after a period of many hours (12, 24, etc., depending on the concentration), and ultimately became and remained wide open. If initially open, they first closed and then reopened. Moderate wilting, such as occurred in the cuttings which were not in water, did not prevent opening.

Greater opening than in the controls was observed in 10 per cent. CO_2. The rate and degree of opening increase with the concentration of CO_2 until the latter becomes toxic. The occurrence of injury depends greatly on the time of exposure, so that no definite limit of concentration can be named. Even 15 or 20 per cent. causes injury after two or three days, while concentrations over 50 per cent. are endured for several hours. The injury, however, is long subsequent to the opening and is not its cause. The cause is increased turgor pressure (up to the equivalent of $\frac{M}{2}$ $CaCl_2$) of the guard cells, due to conversion of starch to sugar, presumably as a consequence of the high acidity.

The effect of increased concentrations of CO_2 in light is usually to prevent opening. An atmosphere in which the gas is present in sufficient concentration to cause opening when in darkness may prevent it in light, no doubt because the CO_2 concentration inside the leaf is reduced by photosynthesis.

In some cases the stomata were found wide open in high CO_2. This might be explained by weakness of the light in these experiments, being insufficient to reduce the CO_2 concentration in the leaf to the closing level.

As regards the effect of a *reduced concentration* of CO_2 in absence of light, this is not easy to test, because if the leaf is intact the gas accumulates inside it through respiration, while if the guard cells are exposed by cutting the wound stimulus inhibits opening. It was found, however, that in guard

cells exposed to CO_2-free water there is much less accumulation of starch than in those exposed to ordinary water. There is even some disappearance of starch and slight opening if the stomata are shut at the beginning.

The fact that it takes several hours for shut stomata to open under the influence of CO_2, probably on account of the time required for the gas to diffuse into the leaf, and the fact that open stomata close to some extent before they finally open, may account for the difference between LINSBAUER'S results and those now presented.

In brief, these preliminary experiments not only confirm the results of LINSBAUER—that increased concentration of CO_2 in the atmosphere may cause closure in light—but also in every case yield the result that beyond a certain concentration CO_2 causes permanent opening in darkness. This concentration is less than would develop inside the leaf if all the oxygen of a normal atmosphere were replaced by CO_2 through respiration.

These results lend support to a suggestion made in a former paper that stomata regulate against excess as well as against deficiency of CO_2 in the leaf, and that opening after prolonged absence of light may be due to accumulation of the gas. It is not necessary, however, to assume that CO_2 is the only acid product of respiration in the guard cells or other cells which is resynthesized and disappears under the action of light, since it has been demonstrated that in succulent plants organic acids accumulate by night and disappear by day. In fact, it is theoretically not improbable that the semianaerobic condition of a leaf which has been kept in darkness for some time will result in incomplete oxidation of carbohydrate and production of organic acids. Whether other acids are produced or not, the relation between stomatal opening and CO_2 concentration harmonizes well with the relation between opening and acidity of the guard cells already noted. The pH of closure is such as could be induced by lower concentrations of CO_2 and that of acid opening by higher concentrations, provided the cells are weakly buffered. Apparently also, absence of CO_2 without the aid of light produces a pH at which hydrolysis will take place. Whether, in addition to this, light stimulates opening in some other way cannot be said, but at least it can do so only with a sufficiently high pH in the guard cells.

Discussion

A. Effect of light vs. darkness

Light effects stomatal movement by means of that portion of its spectrum which is absorbed by chlorophyll and principally through its action upon the green tissue of the leaf. Any direct action on the guard cells is either subsidiary to or conditioned by its action through the medium of the chlorenchyma.

Light operates through its photosynthetic activity. The relation of its action to CO_2 concentration, as determined by LINSBAUER and others and confirmed in the present work, defines to some extent the means by which photosynthesis can and cannot act. In the first place, since light is equally or more effective in absence of external CO_2, its full effect can apparently be exerted when photosynthesis is merely sufficient to overbalance the effects of respiration; accumulation of its products is not essential. In the second place, since a moderate increase of CO_2 causes closure even in light, while oxygen is not reduced inside the leaf as it is in darkness but instead is being liberated, it follows that photosynthesis and respiration respectively must act through regulating the pressure of CO_2 rather than of oxygen. This does not exclude the possibility of other modes of action but proves that variation of CO_2 concentration is an effective one.

As regards the relation of stomatal behavior to partial pressure of CO_2, three phases are to be recognized.

(a) *In relatively high concentrations* there is hydrolysis of starch with increase of osmotic value in the guard cells and stomatal opening. This takes place regularly after several hours' exposure to the necessary CO_2 concentration (15 per cent. (?) and over in darkness). The partial opening which sometimes occurs in an ordinary atmosphere after a period of darkness may fall into this class; that is, it may be due to an accumulation of CO_2 in the leaf. This phase of response to CO_2 concentration does not seem to have been recorded previously. The next two are well known.

(b) *In lower concentration* of CO_2 the opposite changes take place in the guard cells together with stomatal closure. Closure in an ordinary atmosphere in darkness and in an atmosphere richer in CO_2 in light both fall into this category when composition of the internal atmosphere of the leaf is the criterion.

(c) *In darkness and absence of CO_2*, at least so far as this condition is attained when epidermal preparations are exposed to water deprived of CO_2, there is a greater tendency to reduction of starch in the guard cells and opening of the stomata than in similar material exposed to water containing CO_2, even at ordinary atmospheric pressure. The opening is only meager, however, but two factors (the wound stimulus due to cutting and the CO_2 produced within the cells, both of which militate against opening) are not present in an intact leaf engaged in active photosynthesis. Under the latter condition, therefore, we should expect better opening. According to LINSBAUER, absence of CO_2 assists opening in light.

While it is clear from the present results that reduction of CO_2 pressure through photosynthesis is an essential part of the action of light, the complete adequacy of this condition to produce full stomatal opening in

absence of light is difficult to test and has not been proved by these experiments.

The same three phases of stomatal response as previously noted also appear in relation to the H-ion concentration of the guard cells as governed by other penetrating acids than CO_2 and by penetrating alkali. Acetic acid in relatively high concentration, namely, just short of being rapidly toxic, causes opening; in weaker concentration it causes closure and ammonia again causes opening. This relation was described in an earlier paper. It is now shown that under suitable conditions this regulation of carbohydrate balance, turgidity of the guard cells, etc., by artificially changing the pH of these cells, is as ample and complete as in normal movement. It is independent of and overcomes the action of light or darkness, as well as of variations in leaf turgor.

Vital staining tests with indicators point to the conclusion that light and darkness themselves induce wide fluctuations in the pH of the guard cells, extending from about pH 5 after a period of darkness to between pH 6 and pH 7, or perhaps higher, after a period of good illumination. The experiments do not show whether or not it reaches a point (probably about pH 7.3) at which hydrolysis of starch and opening of the stomata can take place without the aid of light. The possibility remains, therefore, that some action on the part of light in addition to changing the pH is involved when complete opening takes place.

The acid range of pH and therefore the behavior in darkness can be accounted for by production of CO_2 alone if the guard cells are not too well buffered; although, of course, other acid products of respiration may assist in developing a low pH.

B. Effect of leaf turgor

When closure is induced by wilting of the leaf, the guard cells exhibit a *low pH* and other characteristics associated with closure in darkness, even though the leaves are exposed to bright sunlight. Considerable wilting does not prevent opening (accomplished by the usual mechanism) in leaves exposed to amomnia gas or to the higher pressure of CO_2. This seems to prove that within limits, scarcity of available water does not prevent hydrolysis of starch in the guard cells, provided their pH is right for the process; and that wilting, like darkness, acts by creating an H-ion condition conducive to closure. It may do this by inhibiting photosynthesis. The effects of leaf turgor both in light and darkness are dealt with more fully in the paper which follows.

C. Effect of temperature

Low temperature tends to produce closure, and high temperature opening of stomata, whether in darkness or in light. Here again, to judge by

vital staining, the movement is correlated with the same pH change as under the influence of the other factors.

In a fuller study of the temperature relation to be described in a subsequent paper, it will be demonstrated that the turgor of the guard cells is regulated by the same carbohydrate transformations as in these cases. The pH difference due to temperature cannot depend on photosynthesis, because it occurs in darkness.

D. Effect of wounding

Injury to cells, *e.g.*, by cutting, causes stomata in the immediate vicinity of the wound to close. In this case the loss of turgor in the guard cells is unaccompanied by increase of starch and is probably due to escape of solute; that is, to increased "permeability."

Summary

1. All major stomatal movements in the plants studied, whether due to change of illumination, of leaf turgor, or of temperature, are accompanied by reversible transformation of carbohydrate and also by pH change in the guard cells.

2. The carbohydrate balance depends strictly on the pH.

3. The origin of the pH change, when governed by the light factor at least, depends mainly on the relative rate of photosynthesis and respiration respectively in the leaf tissue in general and demonstrably on the CO_2 pressure in the environment of the guard cells.

4. Absence of CO_2 favors, if it does not of itself induce, stomatal opening.

5. A certain concentration of CO_2 causes closure; a higher concentration, opening.

6. Any response of the guard cells to the direct action of light is small or else conditioned by the environmental state.

7. An entirely different mechanism, namely, increase of permeability, seems to be involved in the stomatal closure which takes place in the vicinity of a wound.

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COMPARISON OF THE EFFECTS OF TEMPERATURE ON THE RADIAL AND LONGITUDINAL ELECTRIC POLARITIES IN WOOD AND CORTEX OF THE DOUGLAS FIR

E. J. LUND

(WITH FIVE FIGURES)

Introduction

In previous papers it has been shown, chiefly by indirect methods, that all the present known facts about the distribution of correlation potentials in the Douglas fir can be explained by the existence of two oppositely oriented radial E.M.F.s, one of which is located in the wood and the other in the cortex. The radial E.M.F. in the wood increases toward the base while that in the cortex increases toward the apex. The direct demonstration of the separate existence of these two E.M.F.s is not a simple matter, because mechanical removal of the cortex always involves change in its inherent E.M.F.

Since it has been shown that the external polarity of the apex can be increased, decreased, inverted, or made equal to zero by an appropriate change in temperature, it becomes of importance to know how a temperature change affects the two different sets of E.M.F.s in the wood and cortex. The following experiments which bear upon these questions yield further confirmatory evidence for the existence of the two oppositely oriented E.M.F.s. They also point to the conclusion that when the temperature of the tree is changed, the pattern of the internal correlation potentials is changed. This result appears to depend fundamentally upon the general fact that *equal change in temperature changes the E.M.F. unequally in different loci of the axis in polar tissues.* An explanation of this unequal effect has already been presented (LUND **1, 2**).

Procedure

Since nothing is known about the effects of temperature on the internal electric phenomena associated with polarity, the experiments described in the present paper are in a sense exploratory. The plan was to follow nearly simultaneously the changes in the magnitude and direction of the following E.M.F.s in the same stem segment, with the same change in temperature.

1. Longitudinal E.M.F of intact stem.
2. Longitudinal E.M.F of wood axis without cortex.
3. Radial E.M.F. of intact stem.
4. Radial E.M.F. of wood axis without cortex.
5. Diagonal E.M.F. of wood axis without cortex.

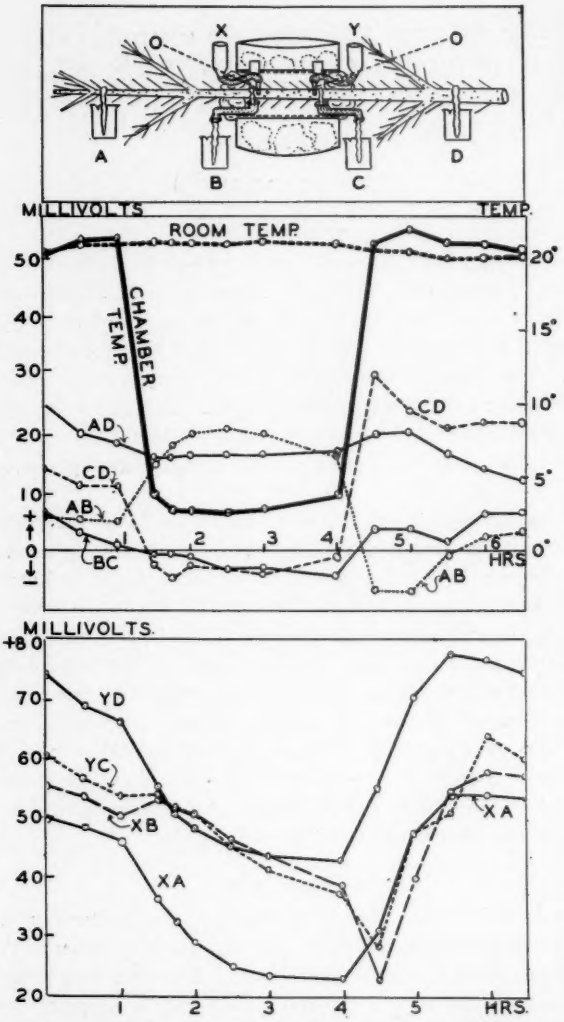


FIG. 1. The diagram at the top shows the arrangement of the apparatus. The cups *X* and *Y* are filled with tap water and connect by rubber tubes and glass cannula to the center of the wood at *O*. The E.M.F. between contacts *X* and *B*, and *Y* and *C* is radial. The third internode was used in the experiment. The distances between the contacts *A* and *B*, *B* and *C*, and *C* and *D* were respectively 22, 12.5, and 19 cm. The lower sets of graphs are separated from those above to facilitate comparisons. Both sets of curves are drawn to the same scale.

6. Diagonal E.M.F. of intact stem.

7. E.M.F.s in dead and living parts of same stem.

For the sake of comparison, the results from four types of experimental procedure will be given. A diagram of the apparatus and contacts used in each of these procedures is given at the top of each of the figures 1, 2, 3, and 5.

Before the results given by the curves are considered, a comparison should be made of the four different experimental arrangements. Note that four contacts labeled *A*, *B*, *C*, and *D* in the figures are made in a similar manner in each of the four types of experiments. Two of these electrode contacts (*A* and *D*) were outside the chamber and remained at room temperature throughout the experiments; the other two external contacts were placed inside the chamber, and together with the stem segment were subjected to the change in temperature.

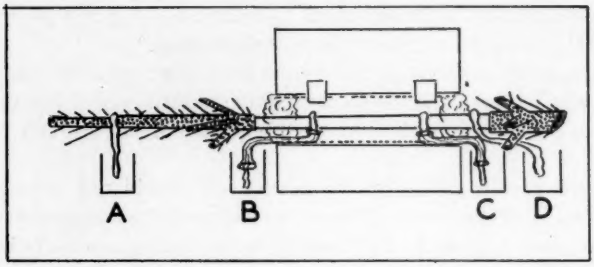
For measurement of the radial and diagonal E.M.F.s two more electrode contacts (*X* and *Y*) were made at the points indicated by inserting a glass tube 2 mm. in diameter to the bottom of a hole drilled exactly to the center of the wood axis. The glass tubes were connected by rubber tubes to cups on the outside as shown in figures 1, 3, and 5. In this way the electrode connection with tap water was made at the center of the wood axis. The duration of the experiments varied from four to seven hours, and as usual all measurements were made with the potentiometer. The first two experiments, given in figures 1 and 2, were made on the third internode from each of the tops of the main stems of two different trees. The second and third experiments, given in figures 3 and 4, were similarly made on the second internode from each of two different trees. The results of the experiments given in the figures are typical of other experiments which will therefore not be reported.

The significance of the results shown in the curves can be appreciated only when a careful comparison is made of the properly selected curves in the different figures.

Longitudinal E.M.F.s in wood and cortex

1. Longitudinal E.M.F. on wood axis is not affected by change in temperature.[1]—This fact is shown in part in curve *BC* of figure 2, which should be compared with curve *BC* in figure 1. Cooling one contact placed on the wood axis does not affect the E.M.F. between it and another contact placed either on the wood axis or on the cortex of another region kept at room temperature. Evidence for this statement is found by comparing curve *AB* of figure 2 with curve *AB* of figure 1, and is also shown by curve

[1] By this statement is meant that within the limits of accuracy in procedure in the present experiments the curves do not show any clearly evident effect.



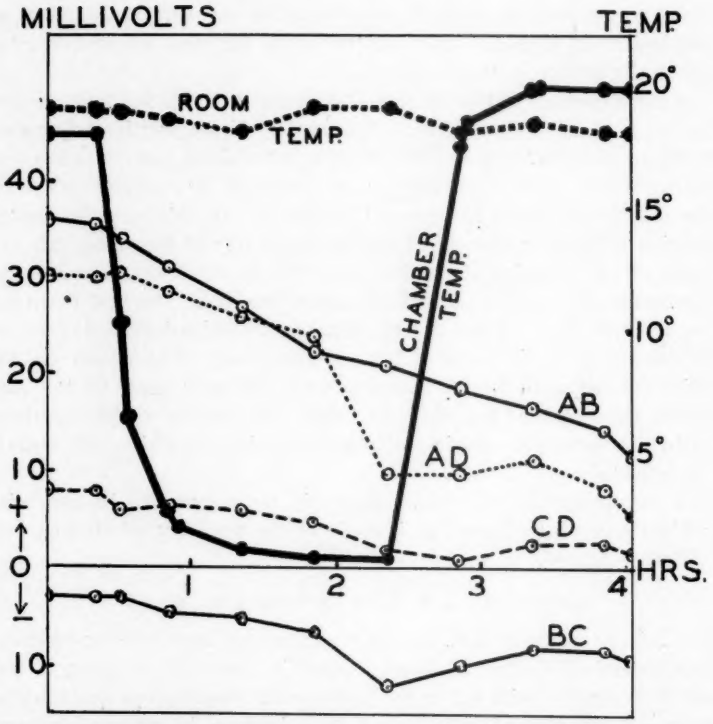


FIG. 2. The third internode from the apex of a tree 25 feet high was used. The cortex was removed over the length indicated by absence of stippling. Contact *A* is on the outside of the cortex; contacts *B*, *C*, and *D* are on the wood axis. Contacts *B* and *C* are subjected to change in temperature; contacts *A* and *D* remain at room temperature. The distances between *A* and *B*, *B* and *C*, and *C* and *D* were respectively 22, 13, and 8.5 cm. The total length of exposed wood axis is 34.5 cm.

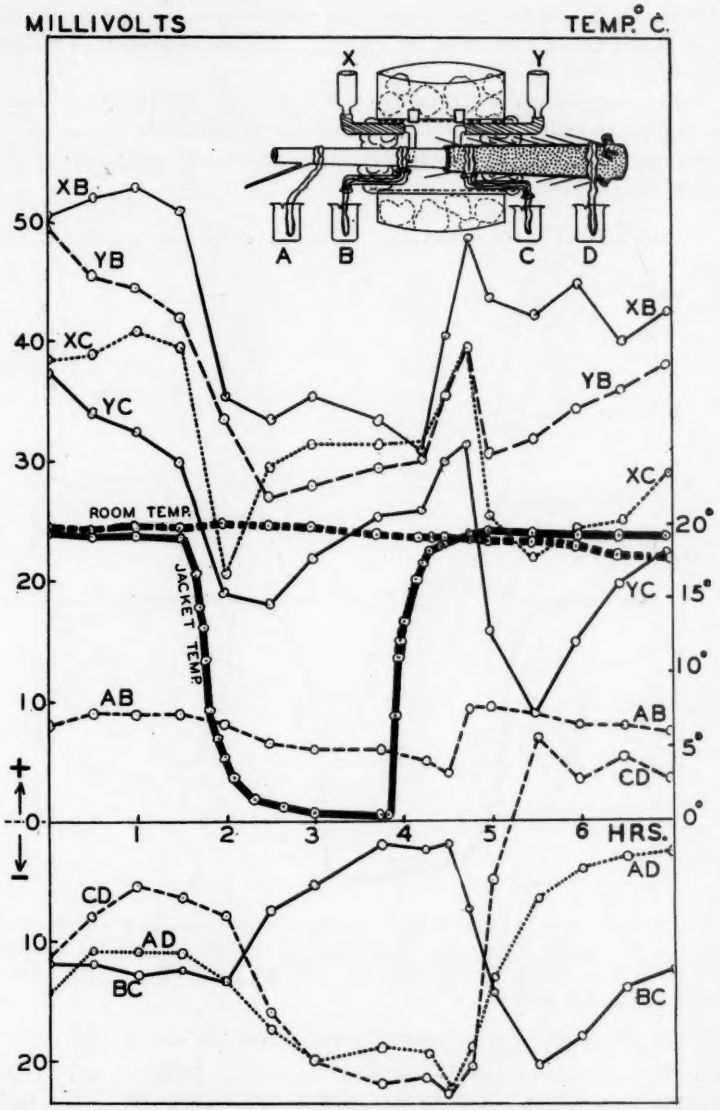


FIG. 3. The second internode from the main stem of a tree was used. The cortex was removed from the unstippled portion of the internode shown in the diagram. Distances between electrodes *A* and *B*, *B* and *C*, and *C* and *D* were respectively 14, 13, and 17 cm.

CD of figure 2. In this latter case both contacts are on the wood axis but one is cooled and the other is not cooled. This curve should be compared with curve *CD* of figure 1.

Again compare curve *AB* in figure 3 with curve *CD* in figures 3 and 1. The sudden bend in curve *AB* of figure 3 is unexplainable as an effect of temperature, and may have been due to an accidental mechanical displacement of the contact.

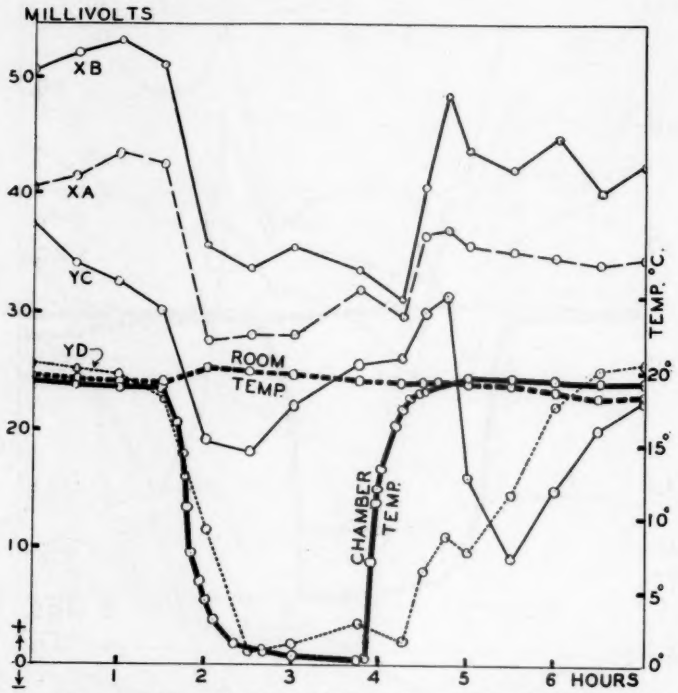


FIG. 4. The curves show the E.M.F.s between the correspondingly labeled electrode contacts in the diagram of figure 3. They are shown separately to facilitate comparison of the effects of temperature. Note that $XB > YC$ and $XA > YD$. The form of curve *YC* is of special significance.

2. LONGITUDINAL E.M.F. BETWEEN TWO CONTACTS ONE OF WHICH IS ON WOOD AND THE OTHER ON THE CORTEX IS CHANGED BY EQUAL CHANGE IN TEMPERATURE OF CONTACTS AND REGION WHICH THEY INCLUDE.—This fact is shown by comparing curve *BC* of figure 3 with *BC* of figure 2. A comparison should also be made between the magnitudes of the effects of temperature in curve *BC* of figure 3 and that in curve *BC* in figure 1. The ap-

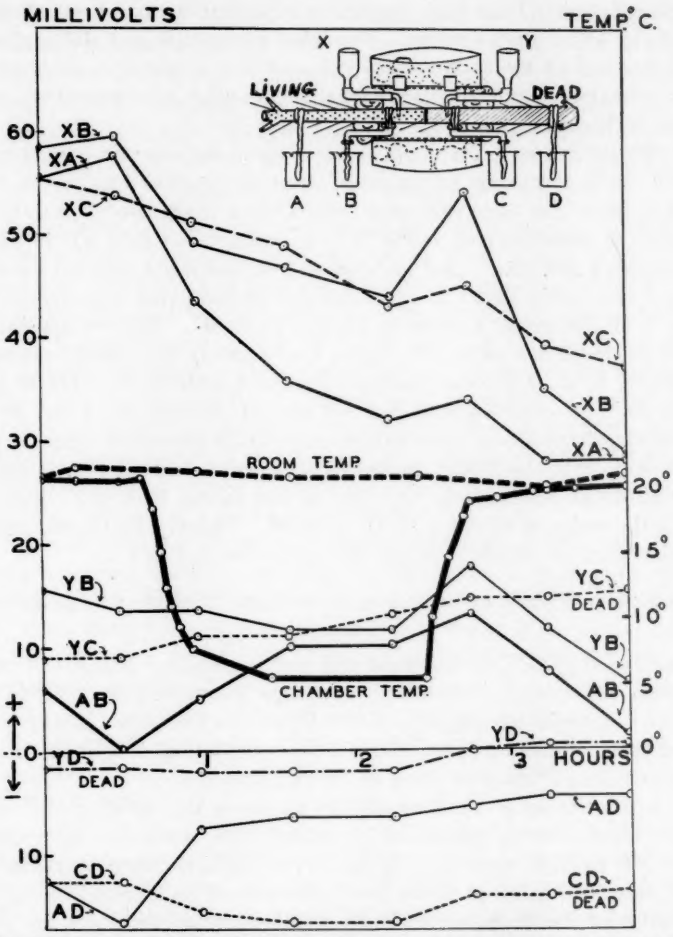


FIG. 5. The curves show the difference between the effects on the E.M.F.s caused by the same change in temperature in a dead and living section of the stem. The second internode of a main stem was used. The basal half of the internode was immersed in boiling water during 4 minutes and then cooled to room temperature, while the apical half was kept cool by wrapping in a cool wet cloth. The distances between the contacts *A* and *B*, *B* and *C*, and *C* and *D* were respectively 15, 14, and 14 cm. The internode was allowed to come to room temperature before the experiment was begun. The E.M.F.s between the contacts *Y* and *C*, *Y* and *D*, and *C* and *D* are only slightly affected by a temperature change which affects markedly the corresponding contacts in the living part of the stem.

parent conclusion from such experiments is that temperature affects the E.M.F. by affecting the cortex. Note that curves *CD* and *AB* of figure 1 and curve *CD* of figure 3 confirm fully all of the corresponding observations presented in the previous paper on the effect of temperature on the E.M.F. in the apex (LUND **2**).

3. WHEN ONE CONTACT IS PLACED ON THE WOOD AND THE OTHER ON THE CORTEX, BOTH CONTACTS BEING KEPT AT SAME TEMPERATURE, THEN WHEN THE REGION OF THE JUNCTION BETWEEN EXPOSED WOOD AND NORMAL STEM IS CHANGED IN TEMPERATURE, THE E.M.F. IS CHANGED.—Curve *AD* in figure 3 demonstrates this fact. This curve should be compared with curves *AD* in figure 2 and *AD* in figure 1. It should also be compared with curve *AF* in figure 2 of the writer's previous paper (LUND **2**). The remarkable fact should be noted that curve *BC*, figure 3, changes in an opposite manner to *AD*, figure 3, when the temperature is lowered around the contacts *B* and *C* and their included segment, but not around the contacts *A* and *D*.

Finally, the effects of temperature as shown in curve *AD*, figure 3, prove conclusively that the E.M.F. measured between contacts *A* and *D* does not all originate at the contacts, and that all the change in E.M.F. does originate in the region of the stem which is cooled. This is a simple but convincing demonstration of the existence of summation of E.M.F.s.

EFFECT OF TEMPERATURE ON RADIAL POTENTIALS IN WOOD AND WOOD-CORTEX SYSTEM

The radial E.M.F. of the wood and the longitudinal E.M.F.s of the intact stem are similarly affected by a change in temperature. This fact is shown for example by the curve *XB* in figure 3. From this it may be concluded that the absence of an effect on the longitudinal E.M.F. of the wood is not due to an absence of effect on the radial E.M.F.

That a lowering of the temperature decreases the radial E.M.F. of the bipolar wood-cortex combination is evident from curve *YC*, figure 3, and curves *XB* and *YC*, figure 1. These curves exhibit interesting characteristics of form which are probably due to the rate of cooling and the opposite orientation of electric polarities in the wood-cambium-cortex system. However, since the present experiments are not intended to show more than some of the more important general characteristics of the effects of temperature, a fuller analysis of the precise course of the effects of change of temperature upon any one type of measurement must be omitted for the present.

EFFECT OF TEMPERATURE ON DIAGONAL E.M.F.S

Curves *XA* and *YD* in figure 1 show a closely parallel effect. It should be observed that these measurements were on the intact stem. Curves *XA* and *YD* in figure 4 should be compared. Note that the E.M.F. of *XA* is

greater than the E.M.F. of *YD*, as it should be if the inherent E.M.F. in the wood and the cortex are oppositely oriented. Note also that this relation in magnitude is not true when curves *XA* and *YD* of figure 1 are compared. This again fulfils expectations, as mentioned later.

All experiments show that lowering of temperature lowers the diagonal E.M.F.s.

Inherent E.M.F. in cortex

The following facts derived from a comparison of the curves show that an inherent E.M.F. exists in the cortex. Curve *AB*, figure 3, and curves *AB* and *CD*, figure 2, show that the longitudinal potential at and in the region of the contact *B* on the wood is not affected by a change in temperature. On the other hand, curves *CD* in figures 1 and 3, and curve *AB* in figure 1 show that changing the temperature on a contact on the cortex and its adjacent region changes the E.M.F. between the contacts. Again, curve *BC* of figure 3 shows that change in temperature affects the E.M.F. between the contacts *B* and *C*, one of which is on the wood and the other on the cortex. But curve *BC* of figure 2, where both contacts are on the corresponding regions of the wood, shows no such effect.

Finally, curve *AD* in figure 3 shows that temperature affects the E.M.F. between these two contacts even though they themselves are not cooled, while curve *AD* in figure 2 shows no such effect of cooling on its E.M.F. It follows from these facts that the cortex in the region *C* in figure 3 is the seat of an inherent E.M.F. whose magnitude is markedly affected by a change in temperature. These facts, when taken in conjunction with certain facts presented in previous papers (**2**), appear to constitute a definite proof that the cortex possesses an inherent E.M.F.

The second question of special interest is, what is the orientation of the E.M.F. in the cortex?

Curve *BC* in figure 3 shows that contact *B* on the wood is electronegative in the external circuit to contact *C* on the cortex. In the same way curve *AB* in figure 2 shows that *B* is electronegative to *A*.[2] Now, since the E.M.F. at the contact *B* and its region on the wood is *not* affected by a change in temperature, as shown by curves *AB* and *CD* in figure 2, while the E.M.F. between contacts *B* and *C* in curve *BC* of figure 3 is changed by a change in temperature, then the E.M.F. between *B* and *C* in figure 3 must lie in the cortex as just shown. Since the surface in contact with *C* is electropositive to *B*, then this must mean that the E.M.F. of the cortex is oriented in such a way that the outside of the cortex is electropositive to the inside of the cortex in the external circuit.

[2] The curve *AB*, figure 2, is shown *above* instead of below the base line in order to comply with the convention adopted in labeling polarities as given earlier in the paper.

Furthermore it is shown in figure 4 that the E.M.F.s of *XB* and *XA* are respectively greater than the E.M.F.s of *YC* and *YD*. This relation is never observed in the intact stem, such as that in figure 1 where $YD > XA$ and $YC > XB$. These differences can be explained if we assume that an E.M.F. exists in the cortex, this E.M.F. having an opposite orientation to that in the wood. These facts again indicate the same conclusion as that arrived at in a previous paper (LUND **4**) by different methods of experimentation.

The results suggest that the longitudinal E.M.F.s observed when the leads are attached to the intact outer surface of the cortex are probably mostly derived from the inherent E.M.F. in the cortex. This conclusion seems to be strengthened by the demonstrated fact that the external polarity in the apical region is much greater than that in the more basal regions, in spite of the fact that the thickness of the wood and therefore the radial E.M.F. in the wood diminishes to small value in the apex.

Finally it may be recalled, as shown in a previous paper (**4**), that the diagonal basal-apical (inside to outside) E.M.F. in the apex is oriented oppositely to that in the lower parts of the stem.

All the diverse facts seem to leave no doubt that the inherent E.M.F. in the cortex is oriented oppositely to that in the wood. This conclusion agrees of course with the morphological and functional bipolar character of the cambium.

General conclusions

With the possible exception of the external longitudinal E.M.F.s on the isolated wood axis, all the observed E.M.F.s are markedly affected by a change in temperature. In all of these cases it appears that when the temperature of both electrode contacts and the whole region of the stem involved in the measurement of the E.M.F. is lowered, the observed E.M.F. decreases. In other words, all the observed results appear to be due to the simple but apparently fundamental fact that *equal* change in temperature causes an unequal change in the E.M.F.s along the polar axes, whether these be longitudinal, radial, or diagonal resultants of the elementary polarity potentials of the living cells. This principle reminds us of the similar unequal effects on the velocity of oxidation in apical and basal ends of the stem of *Obelia*, produced by equal change in oxygen concentration.

The present experiments indicate that when the temperature of the tree system is changed uniformly throughout, or when one part of it is subjected to a change in temperature which is different from the temperature maintained in other parts, the *relative magnitude* and even the *directions* of flow of the electric correlation currents must become changed in a definitely

characteristic manner. The possible consequences associated with such a change in the pattern of the electric correlation mechanism appear now of paramount interest. Experiments will deal with these problems in the papers which are to follow.

Comparison of effect of change in temperature upon the living and dead stem

Only one experiment is given to show the typical differences in behavior of the E.M.F.s in a dead and in a living stem. The results are given in figure 5. In this experiment the readings of E.M.F. were made at longer time intervals and therefore the curves do not bring out the differences as clearly as they otherwise would have done.

The basal half of the second internode of the main stem of a tree was killed in boiling water, cooled to room temperature, and set up in the apparatus as shown in the diagram at the top of figure 5. After allowing the preparation to rest for one-half hour, the readings of E.M.F.s were taken as shown in the curves.

The first fact to notice is that E.M.F.s exist in the dead half of the stem just as we might expect to find in a telephone pole, chimney, or between two sides of many other objects. An intelligent appreciation of the facts, however, leads to the conclusion that the behavior of the E.M.F.s in the dead part of the stem when subjected to change in temperature is radically different from the corresponding ones in the living stem. This fact is shown by a comparison of the effects of temperature on the radial potentials, curve *YC* which is dead and curve *XB* which is living. Similarly, curve *XA* (living) and curve *YD* (dead) should be compared. Curve *CD* (dead) is to be compared to curve *AB* (living). The general fact observed in the curves is that whenever both contacts lie in and include only dead stem between them, the E.M.F. is not affected by temperature, except to a small extent as would be expected if they are concentration potentials of various types in a non-living system. This is shown by curves *YD*, *CD*, and *YC* in figure 5.

Summary

1. The longitudinal and radial E.M.F.s in the intact stem below the apex of the Douglas fir are both greatly decreased by lowering the temperature and increased by raising it. The radial E.M.F. of the isolated wood axis is affected in a similar manner. Dead stems often show E.M.F.s but these are not affected by temperature like those in the living stem.

2. By changing nearly simultaneously the temperature of the wood and cortex, it is possible to show that the cortex is the seat of an electric polarity oriented in the opposite direction to that in the wood. The radial E.M.F.

in the cortex appears to be equally or more sensitive than that in the wood to the same change in temperature.

3. The same change in temperature causes a greater change in the E.M.F. of the cortex in apical regions than in the cortex of relatively more basal regions.

4. The phenomenon of "rebound" of the E.M.F. after a period of exposure to low temperature is less pronounced in lower regions of the stem than in the apex. Additional evidence shows that summation of E.M.F.s occurs.

5. All of the observed effects of temperature on the various polarities in the living tree appear to be due to the simple but apparently fundamental fact that equal change in temperature causes an unequal change in the E.M.F. along the polar axis under consideration. In all cases the region having the highest relatively electropositive condition with respect to the external circuit is changed by the greatest amount.

6. Temperature acts as one of the primary environmental factors which can control the magnitude and even the direction of the internal electric polarities in the tree system. Change in temperature can and does change the pattern of the internal electric correlation currents in the Douglas fir.

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DETERMINATION OF THE INORGANIC NITROGEN IN THE CORN PLANT BY THE EXPRESSED SAP METHOD

R. W. GERDEL

Introduction

In the course of some studies on the chemistry and physiology of the corn plant, it was found by SAYRE and MORRIS (**1**) that the total sugar content could be determined from the analysis of an aliquot of sap expressed from the ground tissue. Since the determination of the inorganic nitrogen content of corn plants grown on plots to which nitrogen fertilizer was applied in several forms and amounts, and by several methods of application, was a part of the problem under investigation, an opportunity was afforded for determining the application of the expressed sap method to the determination of the total inorganic nitrogen.

Material and methods

The sap or juice of the plant was obtained by subjecting 100 gm. of fresh material, previously ground through the medium knives of a hand meat grinder, to a pressure of 5000 pounds per square inch by means of a small laboratory hydraulic press. The green and dry weights of the press cake and the specific gravity of the sap were recorded. The percentage of total solids in the sap was determined by the refractometer, and the weight of sap was calculated by subtracting the green weight of the press cake from the weight of the original sample, in this case 100 gm.

The aspiration method of SESSIONS and SHIVE (**4**) appeared to be best adapted to the determination of the inorganic nitrogen content of the expressed sap. A slight modification of the apparatus was made, 300-cc. Kjeldahl flasks being substituted for the Florence flasks used in the original procedure. The rounded bottom of the Kjeldahl flask permitted the aspiration tube to come in closer contact with the Devarda's alloy, and consequently more of the alloy was held in suspension. The long neck of the Kjeldahl flask also provided more room for the expansion of the foam which occurred with sap from leaf tissue. This foaming was not entirely suppressed by the use of either paraffin oil or caprylic alcohol. The ammonia evolved through aspiration was determined by the boric acid method of SCALES and HARRISON (**3**). The large absorption tubes as used by SESSIONS and SHIVE each contained 50 cc. of a 4 per cent. boric acid solution and 0.3 cc. of brom-phenol-blue indicator.[1] The ammonia was titrated directly with

[1] Preparation of brom-phenol-blue indicator: 0.4 gm. brom-phenol-blue heated with 12 cc. N/20 NaOH until dissolved and diluted to 500 cc. with distilled water.

N/20 sulphuric acid. No loss of ammonia could be detected through the use of the 4 per cent. boric acid solution under the slightly reduced pressure occurring in this apparatus. Since the concentration of the boric acid solution was only approximate, a considerable saving in labor resulted, due to the necessity of preparing only one standard reagent. Titration by reflected light from a 100-watt daylight bulb produced a very sharp end-point with brom-phenol-blue indicator.

The formulas for calculation of results are developed from the basic formula used by SAYRE and MORRIS (**2**). Using figures obtained in this

TABLE I

METHOD OF CALCULATING RESULTS

WEIGHT FIELD SAMPLE			WEIGHT PRESS CAKE FROM 100 GM. LAB. SAMPLE		SAP FROM STALK TISSUE			
LEAVES	STALK	TOTAL	GREEN	DRY	WEIGHT	SP. GR.	TOTAL SOLIDS	N IN 10 CC.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		*per cent.*	*gm.*
2135	2162	4297	21.7	11.7	78.3	1.046	11.85	0.00196

study (table I), this basic formula has been adapted to the determination of total inorganic nitrogen as follows:

$$\text{Per cent. } H_2O = \text{weight lab. sample} - (\text{per cent. total solids} \times \text{weight sap} + \text{dry weight of press cake})$$

$$79 \text{ per cent.} = 100 - (0.1185 \times 78.3 + 11.7)$$

$$\text{Total } H_2O \text{ in stalk} = \text{per cent. } H_2O \times \text{stalk weight}$$

$$1709 \text{ gm.} = 79 \text{ per cent.} \times 2162$$

$$H_2O \text{ content of expressed sap aliquot} = (\text{vol. of aliquot} \times \text{sp. gr.}) - (\text{vol. of aliquot} \times \text{per cent. total solids})$$

$$9.27 = (10 \times 1.046) - (10 \times 0.1185)$$

$$\text{Total inorganic N in stalk} = \frac{\text{gm. N in aliquot}}{H_2O \text{ content of aliquot}} \times \text{total } H_2O$$

$$0.3615 \text{ gm.} = \frac{0.00196}{9.27} \times 1709.$$

Experimentation

AMOUNT OF REAGENTS REQUIRED

In the preliminary stages of this study, considerable variations in the nitrate nitrogen content of duplicate samples were found. This excessive variation between duplicates was overcome by the use of an additional 2.5 gm. of Devarda's alloy and 5 cc. of 6 per cent. NaOH, and by a longer aspiration period, as shown in tables II and III.

TABLE II

AMOUNT OF REAGENTS REQUIRED FOR COMPLETE ASPIRATION OF NITRATE NITROGEN IN 10 CC. CORN SAP BY SESSIONS AND SHIVE METHOD

SAMPLE	NITRATE NITROGEN RECOVERED						TOTALS AVERAGED
	DUPLICATE 1			DUPLICATE 2			
	1ST* RUN	2D* RUN	TOTAL	1ST* RUN	2D* RUN	TOTAL	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	5.93	1.96	7.89	7.28	1.54	8.82	8.355
2	6.96	2.52	9.48	7.77	1.89	9.66	9.570
5	4.76	2.52	7.28	5.60	1.96	7.56	7.420
6	4.48	2.38	6.86	5.60	1.88	7.48	7.170
8	6.23	3.08	9.31	8.19	1.88	10.07	9.690

* 2.5 gm. Devarda's alloy and 5 cc. of 6% NaOH added and aspirated 14 hours for first run; after titration another 2.5 gm. of Devarda's alloy and 5 cc. of 6% NaOH were added and aspirated for another 8 hours.

Note: each 0.1 cc. of N/20 acid is equivalent to 0.07 mg. NO_3 nitrogen.

TABLE III

TIME REQUIRED FOR COMPLETE ASPIRATION OF NITRATE NITROGEN IN 10 CC. CORN SAP BY SESSIONS AND SHIVE METHOD*

SAMPLE	NITRATE NITROGEN				
	DUPLICATE 1			DUPLICATE 2	AVERAGE
	1ST 8 HOURS	2D 8 HOURS	TOTAL	16 HOURS	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	2.10	0.63	2.73	2.59	2.660
2	3.92	1.40	5.32	5.53	5.425
5	2.87	1.12	3.99	3.92	3.955
6	2.73	1.12	3.85	4.20	4.025
8	4.83	1.89	6.72	6.93	6.825
9	2.52	1.26	3.78	3.78	3.780

* 5.0 gm. Devarda's alloy and 10 cc. 6% NaOH added.

Note: each 0.1 cc. of N/20 acid is equivalent to 0.07 mg. NO_3 N.

Apparently, close agreement between duplicate samples may be obtained by the use of 5 gm. of 100 mesh Devarda's alloy and 10 cc. of 6 per cent. NaOH, while an aspiration period of approximately 16 hours appears necessary to remove completely all the inorganic nitrogen in the expressed sap sample.

Recovery of added nitrates

Since in no case, throughout the 1930 season, was the ammonia form of nitrogen found in the corn sap in more than a doubtful trace, studies on the accuracy of the combined "expressed sap" (Sessions and Shive method) were made on inorganic nitrate salts. The efficiency of the method in the recovery of the nitrate nitrogen added as sodium nitrate to both sap and distilled water is shown in table IV.

TABLE IV

Recovery of nitrate nitrogen from expressed sap by Sessions and Shive method

Composition of solution	Total NO_3 N concentration			Total NO_3 N recovered		
	Det. 1	Det. 2	Average	Det.* 1	Det.* 2	Average
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
10 cc. dist. H_2O plus 7.0 gm. N as $NaNO_3$	7.00	7.00	7.000	6.79	6.79	6.79
10 cc. expressed sap	.....	.....	.....	4.62	4.55	4.59
					Total	11.38
10 cc. expressed sap plus 7.0 mg. N as $NaNO_3$	.....	.....	.....	10.99	11.55	11.27

* Each determination is the average of duplicates.

The sum of the averages for the separate determinations of the nitrate nitrogen content of an aqueous solution of sodium nitrate and a 10-cc. aliquot of sap is in close agreement with the nitrate nitrogen content of a 10-cc. aliquot of the same sap to which an equivalent amount of nitrogen as sodium nitrate was added.

Accuracy of expressed sap method

The accuracy of the expressed sap method for the determination of any constituent of the corn plant, which is to be expressed as a part of the whole plant, is dependent upon the proportional quantity of that constituent which will be found in the sap. If the total sap removable from 100 gm. of green tissue is separated into successive aliquots as the pressure on the sample is increased, and if a like amount of the constituent being studied is found in each successive portion of the sap, then it may safely be concluded that the moisture not removable from the tissue at a pressure of 5000 pounds per square inch contains an amount of this constituent proportional to the non-expressable moisture content of the tissue. That such is the case where

the nitrate nitrogen content of the corn plant is being studied is shown by the data in table V.

TABLE V

NITRATE NITROGEN IN SUCCESSIVE PORTIONS OF CORN SAP

PART OF PLANT	1	2	3	4	5	6	AVERAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Leaf tissue, six 10-cc. portions	1.155	1.225	1.155	1.045	1.085	1.085	1.102
Stem tissue, five 15-cc. portions	2.345	2.310	2.275	2.310	2.310	.....	2.310

Note: leaf tissue sample of July 17, stem tissue sample of July 31; all results are averages of duplicate determinations.

There is a close agreement between successive portions of sap obtained from both leaf and stem tissue at increasing amounts of pressure, indicating that the nitrate nitrogen is probably in aqueous solution in the plant and the amount obtained in the sap is proportional to any fraction of the total moisture expressed. Further evidence in support of this hypothesis is shown by the close agreement between the nitrate nitrogen content of the alcoholic extract of an aliquot of ground tissue and the calculated nitrate nitrogen content determined by the expressed sap method on duplicate aliquots of tissue (table VI).

TABLE VI

COMPARISON OF EXPRESSED SAP AND ALCOHOLIC EXTRACTION METHODS

METHOD	NITRATE NITROGEN AS PERCENTAGE GREEN WEIGHT					
	1 A	1 D	14 A	14 D	17 A	17 D
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Expressed sap	0.0268	0.0261	0.0349	0.0531	0.0251	0.0298
Hot alcoholic extraction	0.0266	0.0259	0.0350	0.0517	0.0252	0.0281

PREPARATION OF SAMPLES

When samples consisting of a great number of corn plants must be prepared for analysis, care must be taken in the proper selection of an aliquot from which the sap is to be expressed. The nitrate nitrogen concentration of various portions of the corn plant and the percentage of nitrate nitrogen found in each portion in relation to the total in the plant are shown in table VII.

The stalk or stem of the corn plant was found to contain 76 per cent. of the total nitrate nitrogen on September 9, while the leaves and sheath con-

tained only 3.75 per cent. Not only was the stalk higher in nitrate nitrogen content than any other part of the plant, but the lower part of the stalk (in this case representing everything from the second node below the ear to the ground) contained 57 per cent. of the total nitrate nitrogen found in the plant. Since the concentration of nitrate nitrogen on a percentage green weight basis exhibits considerable variation in separate parts of the plant, the employment of careful technique in the preparation of the sample is important.

The procedure in this study was to prepare the leaves and stalks separately. Sufficient leaves were ground to provide the 100-gm. sample for the hydraulic press. The stalks were split lengthwise into quarters, five or six quarters from as many different stalks constituting a sample for grinding. After thorough mixing, the 100-gm. samples for the press were weighed from this ground material. Although the seasonal variation in the nitrogen content of the ear was not studied as a part of this problem, a procedure similar to that used for preparing the stalk samples was followed in the determination of the data presented in table VII. When studies are to be

TABLE VII

NITRATE NITROGEN IN SEPARATE PARTS OF PLANT; SAMPLED SEPTEMBER

	NITRATE NITROGEN						
	LEAF AND SHEATH			STALK			
	LEAVES	SHEATH	TOTAL	UPPER	MIDDLE	LOWER	TOTAL
NO_3 N per plant portion (mg.)	3.63	2.09	5.72	3.18	25.88	87.00	116.06
Percentage NO_3 N per plant portion (green weight)	0.00312	0.00252	.....	0.01082	0.02931	0.08987	.....
Percentage total NO_3 N in plant	2.38	1.37	3.75	2.09	16.99	57.10	76.18

EAR					SUM TOTAL	STALK		
HUSK	SHANK	COB	GRAIN	TOTAL		NODE	INTERNODE	TOTAL
7.49	1.50	9.27	12.12	30.38	152.16	6.55	18.05	24.60
0.00676	0.00750	0.00728	0.01600	.....	.....	0.03195	0.02282	.....
4.92	0.99	6.09	7.97	19.97	99.90	26.61	73.39	100.00

made between varieties of corn or on corn receiving different fertility treatments, stalk analysis alone may give sufficient data to indicate any variation in the nitrate nitrogen content of the plant.

When treatments or varieties are replicated several times and samples are collected from the replicated material, it appears necessary to collect all samples from like replicates on the same day. Samples which were collected from replicate plots two days apart showed relatively great difference in nitrate nitrogen content compared with the total amounts present, as shown in table VIII; while the variation between similar treatments sampled on the same day was relatively much smaller.

TABLE VIII

METHOD OF SAMPLING TWO OR MORE REPLICATES

PLOT NO.	NITRATE NITROGEN PER PLANT			
	REPLICATE A SAMPLED SEPT. 16; D SAMPLED SEPT. 18		BOTH REPLICATES SAMPLED SEPT. 23	
1 A	23.5		36.1	
1 D	15.8		35.9	
Average	19.65		36.0	
Difference		7.7		0.2
14 A	19.2		72.3	
14 D	7.3		84.5	
Average	13.25		78.4	
Difference		11.9		12.2
17 A	12.4		32.0	
17 D	12.1		35.8	
Average	12.25		33.9	
Difference		0.3		3.8

Variations in climatic factors may be great within a few days' time. Considerable rainfall between September 18 and 23 probably accounts for the great increases in the nitrate nitrogen content of the later samples from all plots. Light rainfall following a long dry season apparently caused the increase in the nitrate nitrogen content of the "A" replicates from the earlier sample date. The rainfall probably was insufficient to benefit the corn for more than a day or two; consequently the "D" replicates sampled

TABLE IX

EFFECT OF FREEZING AT −20° C. AND TOLUENE PRESERVATION ON NITRATE NITROGEN CONTENT OF CORN SAP

PLOT NO.	FRESH				FROZEN − 20° C.				TOLUENE				D_A*
	1	2	AV.	DIF.	1	2	AV.	DIF.	1	2	AV.	DIF.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
1	8.26	8.61	**8.44**	0.35	8.40	8.47	**8.44**	0.07	8.40	8.54	**8.47**	0.14	0.03
2	8.12	8.19	**8.16**	0.07	8.12	8.12	**8.12**	0.00	7.98	8.26	**8.12**	0.28	0.04
5	6.16	6.30	**6.23**	0.14	5.88	5.25	**5.57**	0.63	5.88	6.09	**5.99**	0.21	0.66
6	7.14	6.79	**6.97**	0.35	8.05	7.91	**7.98**	0.14	7.35	7.42	**7.39**	0.07	1.01
8	6.58	6.51	**6.55**	0.07	7.28	7.42	**7.35**	0.14	.....	.....	.....	.....	0.80
9	7.63	7.84	**7.74**	0.21	7.14	7.14	**7.14**	0.00	7.14	7.28	**7.21**	0.14	0.60
1A	8.19	8.26	**8.23**	0.07	8.19	8.19	**8.19**	0.00					0.04
2A	8.47	8.02	**8.25**	0.45	8.26	7.84	**8.05**	0.42					0.20
5A	5.67	5.74	**5.71**	0.07	5.74	6.09	**5.92**	0.35					0.21
6A	5.25	5.32	**5.29**	0.07	5.25	5.46	**5.36**	0.21					0.07
8A	5.46	5.18	**5.30**	0.24	5.25	5.11	**5.18**	0.14					0.12
9A	6.72	7.07	**6.90**	0.35	6.58	6.65	**6.62**	0.07					0.28
14A	6.79	6.57	**6.68**	0.22	6.44	6.65	**6.55**	0.21					0.13
17A	5.53	5.32	**5.43**	0.21	5.46	5.39	**5.43**	0.07					0.00
19A	5.81	5.95	**5.88**	0.14	5.74	5.88	**5.81**	0.14					0.07
20A	6.72	6.72	**6.72**	0.00	6.44	6.79	**6.62**	0.35					0.10

* D_A = difference between extremes of averages.

two days later were lower in nitrate nitrogen content than the "A" replicates which were sampled on September 16.

Preservation of samples

The fact that apparatus sufficient to determine the nitrate nitrogen content of a considerable number of samples at one time is not usually available and would be cumbersome to operate makes the preservation of the sap samples necessary. That both toluene preservation and freezing of the sap at – 20° C. were satisfactory is shown in table IX. Ten-cc. aliquots of sap were pipetted into test-tubes and either frozen at once or preserved in an iced refrigerator after the addition of a few cubic centimeters of toluene to the sample.

The first six samples (plots 1 to 9, table IX) were preserved for one week; the next ten samples (plots 1A to 20A) were preserved for 12 days. Although there is little difference in favor of either toluene or freezing preservation of the samples, the freezing method under long storage would probably be the more satisfactory. Furthermore, the frozen samples are more easily handled, since a slight warming of the test-tube causes the sample to slip easily into the Kjeldahl flask and very little sap remains to be rinsed from the tube.

Summary

1. The Sessions and Shive aspiration method has been found to be satisfactory for the determination of inorganic nitrogen in plant sap. Slight modifications in quantity of reagent and time of aspiration are necessary for the determination of the inorganic nitrogen content of corn sap.

2. The total inorganic nitrogen content of the corn plant can be determined from an analysis of an aliquot of expressed sap.

3. Because of the extreme variation in nitrate nitrogen content of the separate parts of the corn plant, proper preparation of the sample for analysis is important.

4. Sampling of a series of replicates should be done on the same day to avoid variations in the nitrate nitrogen content caused by changes in climatic factors.

5. Expressed sap samples can be preserved for a time by the use of toluene or by freezing at – 20° C. The latter method is preferred.

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RELATION OF HYDROGEN-ION CONCENTRATION OF SOILS TO THE GROWTH OF CERTAIN PASTURE PLANTS

H. P. Cooper

In the course of a study of the ecological conditions influencing the various plant associations found in 546 New York pastures, a large number of pH determinations were made with the quinhydrone electrode. The soil samples were taken from under a number of common species of plants found in these pastures.

It was observed that there is a more or less definite succession of pasture plant associations accompanying the depletion of pasture soil. The types of plants dominating at the various soil fertility levels are also greatly influenced by the climatic conditions as well as by the soil characteristics.

When the timber is first removed from virgin soil, conditions are favorable enough for the growth of Kentucky bluegrass and white clover on practically every well drained soil type in the state. There is usually sufficient nutrient material in the organic residues of the soil for the dominance of crops, like Kentucky bluegrass, which require a high fertility level. The duration of a good Kentucky bluegrass sod in pastures is generally determined by the inherent fertility of the soil.

In the strongly acid soils the available nutrients which have accumulated in their surfaces are soon exhausted and Kentucky bluegrass, which requires a relatively high fertility level, is succeeded by plants tolerant of a lower fertility level. The succession of plant associations accompanying the depletion of pasture soils in regions of New York with cool moist growing seasons may be arbitrarily grouped as follows: (a) Kentucky bluegrass and white clover; (b) Kentucky bluegrass, bent grasses, and white clover; (c) bent grasses and white clover; (d) bent grasses, sweet vernal grass, and white clover; (e) sweet vernal grass; (f) sweet vernal grass and poverty grass; (g) poverty grass; and (h) herbaceous weeds, shrubs, and trees (**1, 3, 4**).

The pH values of soil samples taken from under the vegetation found at the various soil fertility levels are found in table I. Numerous attempts have been made to correlate plant growth with the reaction of soils (**7, 8**). Extended bibliographies will be found in previous papers on this subject (**1, 3, 4**). It is observed that there is no very close correlation between the dominance of various pasture plants and soil reaction. The modal class for Kentucky blue, bent, sweet vernal, and poverty grasses is the same, having a pH range from 5.00 to 5.50. This would suggest that there is some major soil condition other than acidity determining the succession of plants accompanying the depletion of certain soils.

TABLE I

THE pH VALUES OF SOIL SAMPLES UNDER COMMON PASTURE PLANTS FOUND IN NEW YORK

PLANT	pH RANGE AND FREQUENCY DISTRIBUTION										TOTAL
	3.50–3.99	4.00–4.49	4.50–4.99	5.00–5.49	5.50–5.99	6.00–6.49	6.50–6.99	7.00–7.49	7.50–7.99	8.00–8.49	
Kentucky bluegrass											
(*Poa pratensis*)	.....	14.0	42.0	75.0	48.0	43.0	43.0	45.0	44.0	5.0	359
Per cent. in each class	.....	3.9	11.7	20.8	13.3	11.9	11.9	12.5	12.3	1.4	100
Bent grasses											
(*Agrostis canina, A. tenuis*)	2.0	15.0	177.0	239.0	147.0	44.0	33.0	12.0	13.0	2.0	684
Per cent. in each class	0.3	2.2	25.9	34.9	21.5	6.4	4.8	1.8	1.9	0.3	100
Red top											
(*Agrostis alba*)	.....	.....	5.0	20.0	18.0	6.0	8.0	12.0	7.0	1.0	77
Per cent. in each class	.....	.....	6.4	25.6	23.0	7.7	10.3	15.4	8.9	1.3	100
Sweet vernal											
(*Anthoxanthum odoratum*)	.....	1.0	8.0	20.0	5.0	.....	.....	.....	.....	.....	34
Per cent. in each class	.....	2.9	23.5	58.8	14.7	.....	.....	.....	.....	.....	100
Poverty grass											
(*Danthonia spicata*)	.....	1.0	44.0	61.0	49.0	25.0	9.0	4.0	.....	.....	193
Per cent. in each class	.....	0.6	22.8	31.6	25.4	12.9	4.9	2.0	.....	.....	100
Canadian bluegrass											
(*Poa compressa*)	.....	.....	2.0	21.0	25.0	10.0	8.0	15.0	19.0	.....	100
Per cent. in each class	.....	.....	2.0	21.0	25.0	10.0	8.0	15.0	19.0	.....	100
Red fescue											
(*Festuca rubra*)	.....	.....	8.0	31.0	62.0	31.0	17.0	24.0	16.0	4.0	193
Per cent. in each class	.....	.....	4.1	16.0	32.1	16.0	8.8	12.4	8.3	2.0	100
Creeping bent											
(*Agrostis stolonifera*)	2.0	.....	.....	14.0	14.0	12.0	62.0	61.0	17.0	2.0	184
Per cent. in each class	1.1	.....	.....	7.6	7.6	6.5	33.7	33.1	9.2	1.1	100
White clover											
(*Trifolium repens*)	.....	.....	8.0	13.0	10.0	6.0	6.0	1.0	.....	.....	44
Per cent. in each class	.....	.....	18.2	29.5	22.7	13.6	13.6	2.3	.....	.....	100
Total	4.0	31.0	294.0	494.0	378.0	177.0	186.0	174.0	116.0	14.0	1,868
Per cent. in each class	0.2	1.7	15.7	26.4	20.2	9.5	10.0	9.3	6.2	0.8	100

PEARSALL (5) has suggested that the ratios of the various available cations in the soil may be more important than the hydrogen-ion concentration in determining the distribution of plants. It has been suggested by COOPER *et al.* (1, 2, 3, 4) that there is a possible relationship between plant growth and the quantity and strength of the available cations and anions present in certain soils. They believe that the oxidation-reduction potential of nutrient materials may influence their availability in soils, also the qualitative order of absorption of the common atomic nutrient ions by plants. If these conditions exist, one would not expect a close correlation between the H-ion concentration of the soil and plant growth. It is the opinion of PIERRE (6) that the percentage base saturation of soils is often more important than H-ion concentration in determining the growth of plants on certain acid soils.

It is often difficult to distinguish between *Agrostis canina* and *A. tenuis* in the seedling stage, as they are commonly found in well managed pastures. These species are found growing together in many of the pastures in certain regions of New York, therefore the pH values for these two species are combined. Velvet bent is apparently very much more abundant than Rhode Island bent in some of the better bent grass pastures. Velvet bent is the predominant grass in many of the very best pastures found in the state.

The data in table I show that the soil samples from under Red fescue and Canadian bluegrass were less acid than the soil from under Kentucky blue, bent, sweet vernal, and poverty grasses. Creeping bent grass was not generally found on strongly acid soils. The modal class for the creeping bent grass is pH 6.50–7.00. This condition was not expected when this study was begun. These data suggest that creeping bent is not so tolerant as some of the other species of bent grass to nutritional complexes characteristic of certain acid soils. Some of the good pastures in western and northern New York contain large quantities of creeping bent grass.

The pH values of soils collected from under some of the common plants encroaching upon depleted pastures are found in table II. White cedar and arbor vitae are very common on pasture soils containing limestone. The modal class is pH 6.50–7.00. Tamarack is also found on soils with a relatively low hydrogen-ion concentration. Mosses, mountain laurel, and white pine are frequently found on strongly acid soils. These data suggest that gray birch, hemlock, and spruce are very tolerant of nutritional complexes characteristic of strongly acid soils.

Summary

1. Data on the pH value of 2317 soil samples collected from under some common plants found in 546 New York pastures are reported. There is a

TABLE II

THE pH VALUES OF SOIL SAMPLES FROM UNDER COMMON PLANTS ENCROACHING UPON DEPLETED PASTURES IN NEW YORK

PLANT	pH RANGE AND FREQUENCY DISTRIBUTION											TOTAL
	3.00–3.49	3.50–3.99	4.00–4.49	4.50–4.99	5.00–5.49	5.50–5.99	6.00–6.49	6.50–6.99	7.00–7.49	7.50–7.99	8.00–8.49	
Paintbrush (*Hieracium aurantiacum*)	.....	.....	.....	.....	4.0	10.0	.....	1.0	.....	1.0	1.0	17
Per cent. in each class	.....	.....	.....	.....	23.5	58.8	.....	5.9	.....	5.9	5.9	100
Mosses	.....	.....	9.0	14.0	13.0	3.0	1.0	2.0	2.0	.....	.....	44
Per cent. in each class	.....	.....	20.5	31.8	29.5	6.8	2.3	4.5	4.5	.....	.....	100
Mountain laurel (*Kalmia latifolia*)	.....	.....	18.0	36.0	4.0	5.0	.....	.....	.....	.....	.....	63
Per cent. in each class	.....	.....	28.6	57.1	6.0	7.9	.....	.....	.....	.....	.....	100
Beech (*Fagus grandifolia*)	.....	.....	8.0	7.0	11.0	5.0	1.0	1.0	4.0	7.0	.....	44
Per cent. in each class	.....	.....	18.2	15.9	25.0	11.4	2.3	2.3	9.1	15.9	.....	100
Gray birch (*Betula populifolia*)	4.0	5.0	12.0	17.0	2.0	6.0	.....	.....	.....	1.0	1.0	48
Per cent. in each class	8.3	10.4	25.0	35.4	4.2	12.5	.....	.....	.....	2.1	2.1	100
Arbor vitae (*Thuja occidentalis*) White cedar (*Chamaecyparis thyoides*)	.....	.....	.....	4.0	2.0	11.0	15.0	22.0	6.0	5.0	1.0	66
Per cent. in each class	.....	.....	.....	6.1	3.0	16.7	22.7	33.3	9.1	7.6	1.5	100
Tamarack (*Larix laricina*)	.....	1.0	.....	1.0	2.0	5.0	9.0	1.0	1.0	.....	.....	20
Per cent. in each class	.....	5.0	.....	5.0	10.0	25.0	45.0	5.0	5.0	.....	.....	100
White pine (*Pinus strobus*)	.....	.....	4.0	18.0	21.0	7.0	3.0	4.0	3.0	.....	.....	60
Per cent. in each class	.....	.....	6.7	30.0	35.0	11.7	5.0	6.7	5.0	.....	.....	100
Hemlock (*Tsuga canadensis*)	2.0	2.0	10.0	13.0	7.0	6.0	2.0	.....	.....	1.0	.....	43
Per cent. in each class	4.7	4.7	23.3	30.2	16.3	13.9	4.7	.....	.....	2.3	.....	100
Red spruce (*Picea rubra*)	2.0	.....	6.0	4.0	5.0	1.0	.....	.....	.....	.....	.....	18
Per cent. in each class	11.1	.....	33.3	22.2	27.8	5.6	.....	.....	.....	.....	.....	100
Total	8.0	8.0	75.0	119.0	75.0	61.0	34.0	32.0	17.0	17.0	3.0	449
Per cent. in each class	1.8	1.8	16.7	26.5	16.7	13.6	7.6	7.1	3.6	3.6	0.7	100

more or less definite succession of pasture plant associations accompanying the depletion of pasture soils. Kentucky bluegrass requires a fertile soil, and it is likely to dominate on the good soils. As the soil is depleted, bent grasses or red top encroaches upon the Kentucky bluegrass and may dominate. On further depletion of the soil sweet vernal grass may dominate; and finally poverty grass, weeds, and trees represent the dominant type of vegetation.

2. Data from the various grass plant associations accompanying the depletion of pasture soils do not show a close relationship between the soil reaction and the dominance of the various pasture grasses. The modal class for Kentucky blue, bent, sweet vernal, and poverty grasses is the same, ranging from pH 5.00 to 5.50. These data suggest that there is some major soil condition other than acidity determining the succession of pasture plants accompanying the depletion of certain soils.

3. Creeping bent grass was not found on soil with a high hydrogen-ion concentration, which suggests that it is not so tolerant as some of the other species of bent grass to nutritional complexes characteristic of certain acid soils.

4. Arbor vitae and white cedar are common on pasture soils containing limestone. Mosses, mountain laurel, and white pine are frequently found on strongly acid soils. Gray birch, hemlock, and spruce seem to be very tolerant of nutritional complexes characteristic of certain strongly acid soils.

5. It is suggested that a well balanced supply of available cationic and anionic nutrient materials with relatively high oxidation-reduction potentials is much more effective than the hydrogen-ion concentration of the soil in determining the dominance of various pasture plants.

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Total	449
Per cent. in each class	100
	3.0
	0.7
	17.0
	3.6
	17.0
	3.6
	32.0
	7.1
	34.0
	7.6
	61.0
	13.6
	75.0
	16.7
	119.0
	26.5
	75.0
	16.7
	8.0
	1.8
	8.0
	1.8

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ISOLATION IN PURE CULTURE OF GREEN ALGAE FROM SOIL BY A SIMPLE TECHNIQUE

C. E. SKINNER

There have been several procedures described for the isolation of green algae in pure culture, free from bacteria and molds. Among the ones most often cited are those of CHODAT (3), SCHRAMM (7), and PRINGSHEIM (6). These methods are essentially alike. Some of the material from which the algae are to be obtained is inoculated into an inorganic nutrient solution. After incubation in sunlight and when growth is apparent, a small amount is inoculated into an agarized inorganic nutrient solution which is incubated in the light. After incubation, when green colonies appear, some of these are carefully picked out of the agar and each one inoculated into a flask of inorganic liquid media. This "enrichment culture method" is repeated until all the contaminating forms are eliminated. Then, instead of planting in inorganic media, the colonies dissected from the agar are planted in media containing dextrose. In case no molds nor bacteria develop and none can be found under the microscope, the culture is thought to be free from bacteria and fungi, and can be maintained in a manner similar to bacterial cultures.

This method is not very applicable to soil, as was shown by BRISTOL ROACH (2, 8), since inoculation of the soil into inorganic media allows a few rapidly growing species to predominate, and so only cultures which grow most rapidly in liquid media are isolated, whereas other species, possibly more numerous, may be repressed or overgrown. Thus a true picture of the soil flora is not obtained. BRISTOL ROACH (8) had devised a method to counteract this defect in part, but in the opinion of the writer it is complicated and time-consuming. A few species were isolated by her method; but, after introducing simplifications and modifications, I was able to obtain about fifty soil strains of unicellular and small filament strains free from bacterial or fungal contaminants in the space of about eighteen months. Never more than one hour a day was consumed (exclusive of the time spent in preparing the media, which was done by a technician); often nothing was done for weeks at a time; and twice the cultures were not touched for a period of more than ten weeks. No special equipment was needed. The method in principle is that followed by BRISTOL ROACH. With the modifications, however, the isolation of algae from soil offers fewer difficulties than the isolation of anaerobic bacteria by the usual somewhat similar technique (5). This point is emphasized, since not a few botanists and bacteriologists have expressed the opinion to the writer that the separation of green algae from bacterial contaminants was a feat requiring the greatest refinement of

technique, much patience, and an amount of time greater than most people would wish to give to the work.

The following media were used:

MEDIUM I (DETMER'S, diluted to one-third as recommended by CHODAT 4):

$Ca(NO_3)_2 \cdot 4H_2O$	1 gm.
KCl	0.25 gm.
$MgSO_4 \cdot 7H_2O$	0.25 gm.
KH_2PO_4	0.25 gm.
Distilled H_2O	3000 cc.
0.1 per cent. solution of $FeCl_3$	1 cc.

MEDIUM II (BEIJERINCK 1):

NH_4NO_3	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$CaCl_2$ (anh.)	0.1 gm.
K_2HPO_4	0.2 gm.
Distilled H_2O	1000 cc.
0.1 per cent. solution $FeCl_3$	0.2 cc.

MEDIUM III (BEIJERINCK'S modified):

Like medium II, but KH_2PO_4 substituted for K_2HPO_4.

When a solid medium is wanted, the solutions are solidified with 1.5 per cent. high grade agar agar. About 10 cc. of the medium was dispensed in each of the test-tubes which, for solid media, preferably possess thin walls, since many of them will have to be broken. If Burri tubes are available, they may replace the test-tubes and need not be sacrificed. All solid media are sterilized for a minimum amount of time at 15 pounds' steam pressure to avoid hydrolysis of the agar.

Twelve tubes of agar medium I or II are melted and cooled to 42°–45°. Into the first are added a few drops of a 10 per cent. (approximate) thoroughly shaken suspension of soil in sterile tap water. The first tube is rotated vigorously or agitated with the pipette to mix it. From the inoculated first tube, 1–2 cc. of the agar are added to the second and thoroughly mixed. This is continued throughout the series, diluting each time with 1–2 cc. of the previously inoculated tube, thus diluting the soil suspension in series. The same pipette may be used throughout, if sterile precautions are observed and the pipette is cleaned out each time by sucking the agar back and forth. The tubes are placed in racks and incubated, if possible, on a north windowsill in summer, and in the winter away from the cold of the window but where they get a few hours of direct sunlight; for instance, on a table near a south, east, or west window. After an incubation period of about one

month or more, one or two of the tubes showing isolated, imbedded, green colored colonies is broken and the agar cylinder placed on sterile paper. This cylinder is broken repeatedly rather than cut, as many colonies as possible picked out with a sterile and cooled wire, and each colony inoculated into a tube of medium I or III, preferably the latter. A rather fine wire is used, since it cools more quickly and allows less agar to adhere to it. Although a nichrome wire may be used, platinum is better since it cools more quickly. It is advantageous to get as little of the agar as possible into the solution. These tubes are incubated as before in racks or, if sufficient window space is not available, in tumblers. Other agar tubes may be broken later to obtain slower developing colonies. Each tube of liquid media now usually represents a separate strain of soil algae.

As soon as a green color has developed in the tubes of liquid media, shake agar dilution cultures are again made and incubated. When these show growth, tubes showing a few isolated colonies are selected and the colonies again picked. This time, however, the transplants are put into medium II containing also 0.5 per cent. glucose and 0.2 per cent. peptone. About 22 colonies of each strain are transplanted into this medium and two colonies into inorganic medium I or III.

If bacteria or molds are present in the tubes, growth will be apparent in 2–5 days; if not, the tubes are incubated until algal growth is apparent, when they are transplanted to agarized medium II slants to which 0.5 per cent. dextrose and 0.2 per cent. peptone have been added. If no growth of bacteria or molds becomes evident on the slants, the cultures are probably free from these microorganisms. This may be ascertained by staining the cultures with any bacterial stain and by transplantation to bacteriological culture media. That the algae are pure cultures, free from other species of algae, is probable, but this point may be verified by microscopic examination or by a single cell technique, which is much easier than in the case of bacteria. The purified cultures are maintained on agar slants of dextrose-peptone medium II agar, and any future bacterial or mold contamination may be recognized readily on this medium.

The writer has found that in more than one-half of the trials with the 22 tubes as described, at least one of the tubes was free from bacteria and fungi and showed a growth of algae. However, when all the 22 tubes are contaminated, the two tubes of inorganic solution are allowed to grow in the light, are again diluted out in inorganic shake agar cultures, and colonies are picked and again planted into 22 dextrose-peptone solution tubes and into two tubes of inorganic media. The same procedure is repeated with these until a pure culture is obtained.

In a few cases it has happened that the bacteria which were not eliminated in three or four trials could be repressed by inoculation into media acidified with additional KH_2PO_4. In a few cases the molds could not be eliminated by the methods used and were discarded. Possibly the use of an absolutely inorganic medium with silica gel as the solidifying agent would have been effective. This, however, was not attempted.

Although there is little new in principle in this procedure, it is given to soil biologists in the hope that the simplifications may assist in the isolation of soil algae. Since the writer is not a phycologist, no attempt has been made to identify most of the strains obtained. He will be pleased, however, to supply transplants to any botanist who is interested in the taxonomy of algae.[1] It is generally considered far more difficult to free blue-green than green algae from bacteria, and in this the writer is in hearty accord. So far all such attempts have met with failure, although PRINGSHEIM (**6**) and others have succeeded in isolating these interesting forms.

It might be mentioned that the use of the different media was not for the purpose of providing variety. Solutions near a neutral reaction are used in agarized media so as to prevent hydrolysis of the agar. DETMER'S one-third (medium I) is more acid than BEIJERINCK'S (medium II), but medium III (BEIJERINCK'S modified) is so distinctly acid as to cause a decided hydrolysis (**8**). The acid liquid media are used to depress bacterial growth. Inasmuch as medium I contains no NH_4 salt, it may in some cases be used to repress fungous or bacterial growth, since not all molds or bacteria are able to use nitrates. The BEIJERINCK solutions II and III, containing both nitrates and ammonium salts, allow a more rapid growth of algae than solution I; hence both media are used, depending on the condition of the culture. Many other media may be used, the formulae for which may be found in the papers cited. It must be remembered, however, (1) that while acid liquid media tend to repress bacterial growth, excess acid hydrolyzes agar and thus enhances mold development; (2) that nitrates are often inferior to NH_4 salts for algae and also fail to supply available N for certain molds and bacteria; and (3) that media containing NH_4 salts tend to become acid and those containing nitrates tend to become basic, owing to selective absorption of the nitrogen-carrying ions.

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[1] These cultures have been placed in the museum of Prof. E. G. PRINGSHEIM, German University, Prague II, Viničná 3a, Prague, Czechoslovakia, from whom they should be obtained, if desired.

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SUGGESTIONS FOR GROWING MASS CULTURES OF ALGAE FOR VITAMIN AND OTHER PHYSIOLOGICAL STUDY

M. F. GUNDERSON AND C. E. SKINNER

Pure cultures of algae are of great interest to physiologists. By means of them the non-essential nature of sunlight in the formation of chlorophyll was discovered about thirty years ago. This subject has been reviewed several times.[1] More recently WARBURG and his school have made important contributions on the physiology of photosynthesis by use of pure cultures of algae. PRINGSHEIM and his associates have studied, in algae, transformations of organic substances and other physiological phenomena under controlled conditions. CHODAT and his students very early studied the utilization of carbohydrates by green plants (algae) and the effect of nutrition on their morphology. Indeed one may suggest that no higher plants are so suitable for such varied physiological study as are unicellular algae. All bacteria may be eliminated, a thing next to impossible with larger plants; and varied nutritional, light, heat, and other external factors may be studied, one by one, without the chemical changes in the environment inevitable in the presence of bacteria. Then too, such small plants allow one to study large populations and the factor of individual variation ceases to operate. The technique used is essentially bacteriological, and one not trained in such a technique should not expect immediate success. As was emphasized in the preceding paper, however, the technique is not difficult.

The writers wish to suggest another use of pure cultures of algae. Mass cultures of a bacteria-free growth may be grown on a substrate of known chemical composition in light or in darkness. Nutritional or external factors may be varied individually. These mass cultures may be fed to animals on diets and the presence or absence of vitamins determined. The substrate, of course, should be free from the vitamin being studied. Thus the factors necessary for the formation of the various vitamins may be determined. The factors of light and chlorophyll may be separated, since many species of algae form abundant chlorophyll in total darkness in the presence of organic sources of energy. Other factors may also be studied one by one. By introducing certain chemicals or nutrients into the medium one may possibly obtain an insight into the chemical nature of the vitamin itself. Such a study has been initiated at the University of Minnesota, and the first results, now ready, will appear in a later publication. Non-chlorophyllous plants (bacteria) are now being used in the same way.

After various trials, the following method was used for obtaining mass cultures for feeding, and is described in the hope that others may be spared the labor which the writers spent before the method was finally adopted. Growth obtained in the same manner may be used for chemical analysis,

[1] SKINNER, C. E., and GARDNER, CLARA G. The utilization of nitrogenous organic compounds and sodium salts of organic acids by certain soil algae in darkness and in the light. Jour. Bact. **19**: 161–179. 1930.

but it should be remembered that a slight amount of the substrate is obtained with the growth samples.

One hundred and fifty to two hundred cc. of the agarized medium desired are dispensed into one-liter flat medicine bottles. For algae the writers used BEIJERINCK's solution,[2] agar with the addition of 0.5 per cent. glucose. They are sterilized in the autoclave for the minimum time necessary and are allowed to harden after being laid on their sides on a perfectly flat surface. It was found convenient to prepare the media in 10-liter amounts. The agar should be washed repeatedly with alcohol and ether, and the glucose with ether.

After hardening, the flasks are inoculated by adding with a sterile 10-cc. pipette about 5 cc. of a suspension of growth, scraped aseptically from an agar slant into a flask of sterile 1 per cent. NaCl or tap water. It is much more convenient for two persons to do this, one to unplug, flame, and plug the flasks, and the other to do the inoculation. The liquid is shaken over the surface of the medium and the bottles are then laid on their sides in a dark cupboard or incubator. After a little practice, contaminations are infrequent. When growing algae or pigmented bacteria, contaminants can be recognized without difficulty.

After sufficient growth has taken place, the mass of living organisms is washed from the agar. A little water is added and the growth separated from the agar by introducing a brass rod with a flattened end bent at right angles and with rounded edges to avoid cutting into the agar. This growth is washed into large cups which are centrifuged, and the sediment is collected in glass dishes. The supernatant liquid is used again and again in washing off more algal growth from the other flasks. The combined sediment is then dried by directing a current of warm air directly on the growth. It is important that this be done fairly quickly, in order to prevent bacterial growth. Of course, conditions become unfavorable for growth before the algae are completely dry. The final dried growth is ground and used for the purpose desired. If fed as a supplement to vitamin-deficient rats, in the experience of the writers, no difficulty is encountered in getting the animals to eat it. Naturally, all ingredients must be free of the vitamin being studied.

In a similar way bacteria or other microorganisms may be produced in quantity for chemical analyses or for the purpose of feeding. Although more than one rather elaborate and very costly apparatus devised for the purpose has been described, which is much more efficient than the simple method outlined, the writers wish to suggest the above method for those who do not have the more costly apparatus.

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[2] BEIJERINCK, M. W. Notiz über *Pleurococcus vulgaris*. Zent. Bakt. II, 4: 785–787. 1898. (NH_4NO_3, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; $CaCl_2$, 0.1 gm.; K_2HPO_4, 0.2 gm.; H_2O, 1000 cc.; 0.1 per cent. solution $FeCl_3$, 0.2 cc.; agar-agar, 15 gm.).

DETERMINATION OF STARCH IN PLANT TISSUES

R. L. SHRINER

Introduction

During a study of the carbohydrate distribution in grape canes, it became necessary to have a method for the estimation of small amounts of starch. A fairly rapid, accurate method yielding easily reproducible results was desired in order that the analysis of a great number of samples could be completed in a short time.

An excellent survey of the literature dealing with analytical methods for starch is given by WALTON (**11**). Of all the methods listed, one described by THOMAS (**9**) for estimating starch in apple spurs seemed most suitable. The fundamental idea of THOMAS'S method is the direct determination of the combined reducing power of glucose and maltose produced by the action of diastase on starch under prescribed conditions. This method avoids the destruction of sugars which always takes place if complete hydrolysis with acids is attempted or if acid hydrolysis after diastatic conversion of the starch is used according to the Official Methods (**6**). This fact was shown by THOMAS and confirmed in the present work. THOMAS determined the reducing sugars by means of the colorimetric method of BENEDICT and OSTERBERG (**1**), which involved the reduction of a picrate-picric acid solution to picramic acid. When this method was tested out on pure starch it always yielded high results (table I). The use of mercuric nitrate as a clarifying agent was also unsatisfactory since it yielded a precipitate which was slow in settling and difficult to filter. Moreover, mercuric nitrate exerts an oxidizing influence on sugar solutions (**5**). It is evident that the clarifying agent must be chosen to suit the particular type of substrate obtained from the different plants.

The procedure which was found most satisfactory is as follows:

Procedure

Three gm. of finely ground material (50 mesh), which had previously been extracted with 80 per cent. alcohol in order to remove soluble sugars, are heated to boiling on a steam bath for 30 minutes with 200 cc. water, and then cooled to 37° C. An aliquot portion of a solution of taka-diastase is added so that 0.1 gm. of the enzyme preparation is present. Two cc. of toluene are added and the mixture incubated for 48 hours at 37° C. The mixture should be shaken as often as possible during this digestion. The mixture is heated to boiling, filtered, and the insoluble material washed with hot water. One to 3 cc. of a saturated lead acetate solution are added and

the solution diluted to 250 cc. after cooling to 20° C. After shaking thoroughly and allowing the lead precipitate to settle as much as possible the solution is filtered. The excess lead is removed by adding finely powdered disodium phosphate and filtering again. A copper reduction is run on a 50-cc. portion according to the standard MUNSON-WALKER procedure, using the SHAFFER-HARTMANN (8) iodometric method for determining the reduced copper. A blank must be run using 200 cc. water and 0.1 gm. taka-diastase treated exactly like the sample. The difference between the blank and the sample gives the copper reduction value of the digest. This is calculated in terms of glucose from the standard tables. The amount of starch is then obtained as follows:

Calculations

It has been shown (8) that the *ratio* of glucose to maltose in the diastase substrate is 2:1; hence if g = glucose and m = maltose, then $g = 2\ m$ and $m = \frac{g}{2}$ (1).

The average copper reducing value of maltose is 0.61 that of glucose, so if R represents the copper reducing of the diastase substrate expressed in terms of glucose, then $g + 0.61\ m = R$ (2).

By combining equations (1) and (2):

$2m + 0.61m = 2.61m = R$; also $g + \frac{0.61g}{2} = R$

or: $m = \frac{R}{2.61}$ and $g = \frac{R}{1.305}$

Starch value of maltose = 0.947

Starch value of glucose = 0.90

so Sm = starch equivalent from maltose $= \frac{R}{2.61} \times 0.947 = 0.362\ R$

and Sg = starch equivalent from glucose $= \frac{R}{1.305} \times 0.90 = 0.689\ R$

Total starch = Sm + Sg = 0.362 R + 0.689 R = 1.051 R

that is: to determine the amount of starch simply multiply the copper reducing value (R) expressed in terms of glucose by the factor 1.051.

Discussion

The preceding method and calculations are based on the following experimental facts:

1. The presence of glucose and maltose in the diastase substrate is in the ratio of 2:1. When the diastatic conversion is carried out as shown, the variation from the 2:1 ratio is very slight, and THOMAS has shown that

these variations (1.8–2.3) do not materially affect the accuracy of the final percentage of starch.

2. The average copper reducing value of maltose is 0.61 that of glucose (**6**). This value varies somewhat, of course, as is evident from MUNSON and WALKER'S tables and the choice of 0.61 is made purely arbitrarily since it has been found that this value gives good results.

3. The starch equivalents for glucose and maltose are the ones usually given (**9**).

It will be noted that the factor calculated from the experimental data is quite different from the stoichiometric factor calculated from the following equation:

$$\underset{\text{Starch}}{4C_6H_{10}O_5} + 3H_2O \longrightarrow \underset{\text{Glucose}}{2C_6H_{12}O_6} + \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} \quad (3)$$

$$4 \times 162 = 648 \qquad 2 \times 180 \qquad 342$$

Since the average copper reducing value of maltose is 0.61 that of glucose,

then $342 \times 0.61 =$	208
for glucose $2 \times 180 =$	360
sum of glucose and maltose =	568

hence ratio should be $\frac{648}{568} = 1.141$ theoretically.

However, there is no evidence for equation (3). The diastase substrate contains glucose and maltose in the *ratio* of 2 : 1 but this does not mean that four $C_6H_{10}O_5$ units of starch produce two moles of glucose and one of maltose. In fact, the number of moles of glucose and maltose is less than these values and recent work indicates that maltose is not a primary cleavage product of starch but is produced by the synthetic action of the taka-diastase on the glucose. It is also evident from table I that the use of this factor would lead to high results for pure starch. The use of lead acetate as a clarifying agent and disodium phosphate as a de-leading agent is much more satisfactory and more rapid than the use of mercuric nitrate, and it avoids the reduction of any mercury with simultaneous oxidation of the sugars. It is essential that disodium phosphate be used for de-leading, as shown by ENGLIS and TSANG (**4**), and especially so in the present method since the SHAFFER-HARTMANN iodometric titration method for estimating the reduced copper is used. The use of sodium oxalate or sodium carbonate as a de-leading agent is prohibited since both of these interfere with the determination of the reduced copper by this method.

The substitution of the SHAFFER-HARTMANN volumetric method for the colorimetric method has several advantages. In the first place it eliminates

the personal error involved in methods which depend on the matching of colors. The starch-iodine endpoint is easily observed. The titrimetric method is also much more rapid, requiring only one-half to one-third the time required for the colorimetric comparison. Although the objections to and disadvantages of the MUNSON-WALKER reduction method are recognized, it has been found that with a little experience this method yields good results. Since the copper reducing ratio of maltose to glucose is only 0.61 while the picric acid reducing ratio is 0.91, it might be expected that this would result in a reduction in accuracy of the method. The data in table I, in which the present method is compared with other methods on a

TABLE I

DATA ON PURE STARCH

WEIGHT OF PURE STARCH TAKEN	AMOUNT OF STARCH FOUND BY		
	DIASTATIC CONVERSION FOLLOWED BY HYDROLYSIS WITH 0.5 PER CENT. HCl	COLORIMETRIC ACCORDING TO THOMAS	CU REDUCTION ON DIASTASE SUBSTRATE
gm.	*gm.*	*gm.*	*gm.*
0.2000	0.1599	0.2162	0.2008
0.2000	0.1591	0.2162	0.2003
0.3000	0.2531	0.3563	0.2953
0.3000	0.2476	0.3523	0.2961

TABLE II

SUMMARY OF STARCH DETERMINATIONS IN GRAPE CANE

SAMPLE NO.	METHOD		
	BY HYDROLYSIS WITH HCl AFTER DIASTASE COPPER REDUCTION	COLORIMETRIC ACCORDNG TO THOMAS	WRITER'S METHOD
	per cent.	*per cent.*	*per cent.*
1	1.52	2.79	2.46
2	1.98	3.44	2.61
3	3.84	5.44	4.98
4	2.60	3.86	3.43
5	3.25	4.87	3.93
6	3.35	5.29	4.49
7	5.45	5.93	5.30
8	4.27	4.79	4.72
9	1.61	2.63	2.39
10	4.03	5.26	4.50

sample of purified arrowroot starch, indicate that this factor has had no appreciable effect on the accuracy of the results.

It will be noted (table I) that the colorimetric method yielded high results; the acid hydrolysis (0.5 per cent. HCl) after diastatic conversion yielded low results (indicating destruction of the glucose); while the direct copper reduction yielded results closely approximating the amount of starch taken. Since it has been shown that 2.5 per cent. hydrochloric acid possesses a destructive action on glucose (**3, 7, 9, 10**), 0.5 per cent. acid was used (**9**). This concentration is sufficient to hydrolyze the maltose and any remaining unhydrolyzed dextrins. It was also found that the use of 2.5 per cent. hydrochloric acid gave still lower results than those in table I.

In table II the same relationship between the methods is found to hold when applied to the determination of starch in ten different varieties of grape canes.

Summary

A method for the determination of starch is described which involves: (1) conversion of the starch into glucose and maltose by the action of taka-diastase; (2) determination of the combined reducing value of the glucose plus maltose by the SHAFFER-HARTMANN modification of the MUNSON-WALKER method. A simplified method of calculating the amount of starch from these data is given.

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MANNOSE AND THE FIRST SUGAR OF PHOTOSYNTHESIS[1]

HARRY F. CLEMENTS

During the past half century, scientific effort has been made to determine the course of photosynthesis. Among the numerous lines of investigation pertaining to this subject, the occurrence and behavior of the simple sugars and sucrose have interested many investigators. Physiologists seem to agree now that the simple sugars are the first sugars formed by plants; and since the pentoses do not occur generally in leaves, the hexoses, glucose and fructose, might logically be the first sugars formed. It is possible that only glucose is the first sugar synthesized and that fructose, as well as all the other plant carbohydrates, arise from it. If this be the case, then it might well be expected that mannose should be formed also, since structurally it is so nearly like glucose and fructose; and, too, whenever glucose is converted by means of its enolic forms to fructose in the laboratory, mannose is produced as abundantly as fructose. In other words, these three sugars when existing under conditions favoring the enolic conversion will exist in equilibrium. To date, mannose has not been found in photosynthetic tissues, but an attempt to find it there has apparently not been made. It is the object of this study to make a rather extensive search for mannose in leaves of many species of plants. It is hoped that the results may be such as to be of value in determining the first sugar of photosynthesis.

In 1903 HÉRISSEY (3), after studying mannans and galactans in seeds, concluded that these reserves are digested to form mannose and galactose respectively; but since neither of these sugars was positively identified in juices of living plants, if they exist at all they must exist as intermediate products of hydrolysis and must be changed very quickly to other compounds. Thus although mannan, which after hydrolysis yields mannose and mannitol (a hexahydric alcohol), has been found in many plant parts, mannose itself has never been found except in traces in those parts of plants in which the mannan is stored.

The plants used in this investigation were:

Pteridium aquilinum var. pubescens	Pyrus malus
Pseudotsuga taxifolia	Pyrus communis
Abies grandis	Lupinus ornatus
Pinus ponderosa	Pisum sativum
	Melilotus officinalis

[1] Contribution no. 34 from the Botany Department of the State College of Washington.

Pinus monticola
Picea engelmanni
Triticum aestivum
Avena sativa
Tradescantia fluminensis
Allium acuminatum
Veratrum speciosum
Salix sp.
Populus tremuloides var. aurea
Urtica holosericea
Frasera fastigiata
Rumex crispus
Beta vulgaris
Philadelphus lewisii
Heuchera glabella
Rosa spaldingii
Prunus cerasus
Medicago sativa
Geranium viscosissimum
Pelargonium domesticum
Acer negundo
Parthenocissus quinquefolia
Tilia glabra
Althaea rosea
Heracleum lanatum
Syringa vulgaris
Verbascum thapsus
Sambucus coerulea
Balsamorhiza sagittata
Tragopogon porrifolius
Arctium minus
Wyethia amplexicaulis
Helianthus annuus

In this group, the Pteridophyta and the Spermatophyta are represented. Plants belonging to both Gymnospermae and Angiospermae were used. Of the Angiospermae, leaves were collected from plants of 22 families. Not only were plants selected to represent widely separated families, but plants whose soil preferences varied widely were employed.

The leaves of these plants were collected between 1:00 and 2:30 P. M. on successive days, beginning June 12, 1931. Usually six or seven species were taken each day. The leaves were taken into the laboratory and 300 gm. of green material quickly ground in a plate grinder. Sufficient water was added to make the ground mass fairly liquid. The watery material was then brought to a boil and boiled gently for two hours. At the end of this time the liquid was filtered through cheese cloth and cooled. Enough neutral lead acetate was added to clear the solution. After filtration, the excess lead acetate was precipitated with di-sodium acid phosphate and again filtered. The filtrate, which totaled some 300 cc., was reduced under diminished pressure to 20 cc. The pressure was so adjusted that the solution boiled at 35° C. The concentrated solution was cooled under tap water, and whatever precipitate formed was filtered off. To the filtrate, 2.6 gm. of phenylhydrazine hydrochloride and 3.0 gm. of sodium acetate were added. Vigorous shaking of this mixture usually resulted in the complete solution of the added compounds. The samples were then allowed to remain for 24 hours in a refrigerator kept at 1° C. At the end of this period, a search was made for the crystals of mannose phenylhydrazone.

In order to determine the accuracy of the method used, 0.1 gm. of d. mannose was added to 300 gm. of ground leaf material of *Heracleum lanatum, Medicago sativa, Urtica holosericea,* and *Balsamorhiza sagittata* respectively, and thoroughly mixed into each sample. The procedure was followed as just outlined. In each, an abundance of a crystalline precipitate resulted which on recrystallization in 60 per cent. alcohol yielded the platelike, colorless prisms characteristic of the hydrazone of mannose.

Mannose was found to be absent from the leaves of the 42 species of plants used. Since such large quantities (300 gm.) of leaves were used and the extracted solution was concentrated to 20 cc., it is highly improbable that mannose exists even in the slightest traces in photosynthetic tissues, for the method used was found to be sensitive to a 0.03 per cent. solution of mannose.

Although mannose could not be found in these investigations, it may not mean that it is universally lacking in leaves, but it certainly does mean that it forms no part of the general photosynthetic scheme in plants. This being the case, it is probable that fructose in leaves has an origin which is independent of glucose; for unless the stereo-chemical relationships known to exist among glucose, fructose, and mannose are different in plant sap from what they are in pure water, it may be expected that were fructose formed from glucose, mannose should likewise be present. This is not the case.

A number of investigators (**1**) have reported that the occurrence of pentoses is not widespread in photosynthetic tissues. CLEMENTS (**2**) has found sucrose to be absent from leaves during the morning when photosynthesis is active. By a process of elimination, fructose and glucose seem to be the only sugars generally associated with carbohydrate production. That fructose arises from glucose normally in green leaves seems improbable in view of the finding reported herein. Apparently, then, glucose and fructose arise simultaneously as the first sugars of synthesis.

Summary

1. Mannose is found to be absent from leaves of 42 species of plants.

2. Since mannose arises from glucose together with fructose in alkaline solutions, and since mannose is not present in leaf sap, it is concluded that fructose does not arise from glucose in plants but that its origin is simultaneous with that of glucose.

3. By eliminating the pentoses, mannose, and sucrose as sugars not always present when photosynthesis is in progress, it seems probable that glucose and fructose are together the first sugars of synthesis in green leaves.

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BRIEF PAPERS

A PLANT TEST OF THE SUN LAMP[1]

(WITH ONE FIGURE)

In the course of investigations relative to temperature effects in plant growth, it became advisable to adopt artificial illumination as a substitute for difficultly controllable solar heat. This practice involved the issue of spectral adequacy in the case of the ordinary Mazda electric lamp. Tests based upon this point have shown that the young wheat plant is favored in assimilating the nitrate radicle by exposure to the relatively high output of shorter visible and longer ultraviolet radiations emitted by the carbon arc. However, this form is less conveniently controlled for quantitative irradiation than more modern types of lamp.

In view of these considerations it seemed particularly desirable to test the growth responses of plants to the Sunlight (Type S 1) lamp recently developed by the General Electric Company. This lamp is described by LUCKIESCH.[2] A unit of this type was placed at the writer's disposal through combined assistance from the Committee on Radiation of Living Organisms, Division of Biology and Agriculture, National Research Council, and the General Electric Company. Inasmuch as the tomato plant (*Lycopersicum esculentum*) had been found to give rather marked developmental and compositional responses under Vita glass, a form more transparent to solar ultraviolet radiation than common glass, this species was chosen for the present test.

Seedlings of the Livingston Globe variety produced in well manured silt loam were irradiated nine successive nights of 15 hours each, following their emergence from the soil. Sunlight was excluded from the cultures, and the average effective air temperature about the plants was about 22° C. One flat of 60 seedlings was exposed under a 500 watt Mazda C lamp with an intervening cell of flowing water equipped with common glass. A similar flat was exposed to the sun lamp under a water cell constructed from Vita glass. The strongest illumination feasible was utilized, approximating 2000 foot candles. From time to time the distance between the lamp filaments and the upper leaf surfaces was readjusted by the use of a thermopilic photometer to maintain approximate equality of total effective radiation from the lamps.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] LUCKIESCH, M. Artificial sunlight. D. Van Nostrand Co., Inc. 1930.

The appearance of the plants at the close of the test is shown in figure 1. Pigmentation of stem and darker color of leaf under the sun lamp which

FIG. 1. Tomato seedlings after irradiation nine days from emergence: at left, under Mazda lamp; at right, under sun lamp.

were conspicuous here were becoming evident on the sixth day. A rather strong phototropic effect under this lamp was also evident. Yields and partial composition of the upper tissues are presented in table I.

TABLE I

YIELD AND COMPOSITION OF STEMS AND LEAVES IN TOMATO SEEDLINGS WITH RELATION TO IRRADIATION

SOURCE OF RADIATION	WEIGHT OF 60 DRIED PLANTS	LIPIDE CONTENT* OF DRY MATTER		NON-LIPIDE NITROGEN CONTENT OF DRY MATTER	
	gm.	*per cent.*	*gm.*	*per cent.*	*gm.*
Mazda lamp	1.044	3.28	0.034	4.83	0.050
Sun lamp	1.299	2.96	0.038	5.31	0.069

* Ether extract.

Notwithstanding the increased weight of plant under the sun lamp, which might lead one to anticipate more adequate illumination for the requirements of carbohydrate synthesis, the tissue exhibits an increased proportion of nitrogen-containing compounds. This condition is associated with slight depression in the proportion of lipides. As to quantities synthesized, it appears that lipides were favored slightly and nitrogen compounds considerably by exposure of the seedlings to the sun lamp.

Obviously the conditions of exposure solely to the sun lamp are abnormal for plant development. This lamp is intended to provide by relatively brief intermittent exposures the effects of daily exposure to the ultraviolet radiation of sunlight. The results indicate favorable rôles of this form of radiation in relation to the plant. It appears possible to use the sun lamp for limited daily periods in conjunction with major illumination from the Mazda lamp so as to imitate solar radiation of the summer season.—W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin, Madison.*

A DUAL-PURPOSE MICROSCOPE LAMP

(WITH TWO FIGURES)

While in general physiologists make less use of the microscope than do workers in other fields of biology, yet when they are required to make microscopical studies their needs are often extremely exacting. Many times they desire to study cells in the living state, using vital stains or no stain at all where differentiation must depend upon differences in refractive index. Illumination for this type of work must be critical and intensity must be great enough to give bright images after considerable reduction of the condenser aperture.

The writer has constructed a small, compact lamp which has proved very satisfactory for several types of work. It was developed primarily for use with the dissecting microscope (**1**), but has, by the addition of a ground glass and diaphragm and some slight improvements in design, been adapted for use with the compound microscope for both light and dark field work and for photomicrography.

Figure 1 shows the lamp assembled for use and figure 2 shows the various parts. The light source *A*, figure 2, is an Osram 6 volt, 25 watt Nitra globe[1] which fits a standard socket. This is mounted in the rear housing *B* which fits into the front housing *C*. The two condenser lenses *D* and *E* of 5 cm. focal length and 3.8 cm. diameter are mounted in the sleeve *F* by means of the spacer *G* and spring sleeves *H*, and *F* slides into the front tube of *C* and is adjusted by rotating the handle which protrudes through the spiral groove in *C*. A 110 to 6 volt transformer *I* fits in the housing *J*, providing proper current for the Nitra bulb and also serving as a heavy base. The assembled lamp is mounted on *J* by means of a universal joint and swings into several positions, allowing adjustment of height and angle.

The lamp as described thus far makes an excellent illuminator for dissection, providing a beam of intense light. For use with a compound

[1] Obtainable from E. Leitz, Inc.

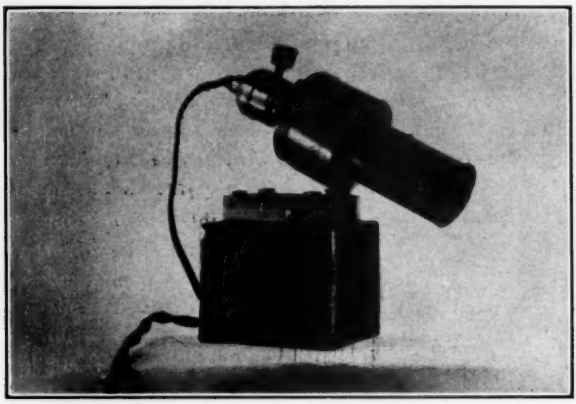

FIG. 1. Microscope lamp with ground glass in position for use with compound microscope.

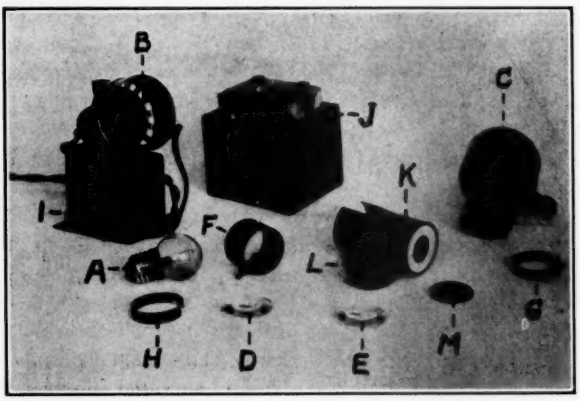

FIG. 2. Microscope lamp disassembled to show the individual parts.

microscope the fitting *K* is slipped on over the front tube of *C*. It bears a ground glass in front of which a hinged door, *L*, with a four-holed diaphragm disk, *M*, may be latched. This disk is pivoted on the back face of *L* and protrudes slightly above it. By brushing the hand lightly along the upper edge of *L* when it is closed, this diaphragm disk may be rotated, centering the different-sized holes with the larger opening in *L*. With the door open and the condenser back, an area 4 cm. in diameter is illuminated for use with the 16-mm. objective. Moving the condenser forward and closing the door *L* gives an intensely illuminated area which may be dia-

phragmed to spots of smaller diameter for use with higher powered objectives. The ground glass was prepared according to directions by BELLING (**2**) and gives a very satisfactory matte.

In using this lamp, it is placed directly in front of the microscope and about 25 cm. from it. The lamp is tilted to point directly at the mirror, and with a slide in place the microscope is brought into focus. Then the condenser of the microscope is adjusted until the outline of the diaphragm on the lamp is in sharp focus in the field of the ocular. The lamp diaphragm should just limit the field of view for low power work, and for very critical high power study it may come considerably within the limits of the ocular diaphragm. If the matte of the ground glass is coarse, the microscope condenser may be slightly raised but the outline of the lamp diaphragm should remain clear.

If a broad color band appears around the edge of the field, the microscope condenser is not achromatic and should be exchanged for one that is. A narrow color band is present with all condensers and serves as a great convenience in centering the microscope condenser. If the latter is out of center this band will vary in color, and by adjusting it so that the color is uniform around the complete periphery of the field, optical centering will be effected.

The addition of a green filter of proper intensity greatly improves this illuminating equipment. The filters are best mounted separate from the lamp so that they can be moved without disturbing the apparatus.

In taking photomicrographs, this lamp may be used either with or without the ground glass. The use of a light filter narrows the transmission band and tends to reduce aberration. The Wratten M filters of the Eastman Kodak Company have proved very satisfactory for this purpose.—A. S. CRAFTS, *Botany Division, University Farm, Davis, Calif.*

LITERATURE CITED

1. CRAFTS, A. S. Some accessories for the dissecting microscope. Plant Physiol. **5**: 430–431. 1930.
2. BELLING, JOHN. The use of the microscope. New York. 1930.

A USEFUL INSTRUMENT FOR MEASURING HEIGHT GROWTH

(WITH ONE FIGURE)

Lack of knowledge of the tools of the machinist often deprives the botanist and forester of the use of standard stock instruments which are well adapted to their needs. This lack causes them to use makeshift substitutes, or to have "special job" instruments made, which is usually an expensive procedure.

In measuring the height growth of trees at frequent intervals during the past growing season, the writer found a standard machinist's tool known as a drill point gauge to be very useful and satisfactory. The drill point

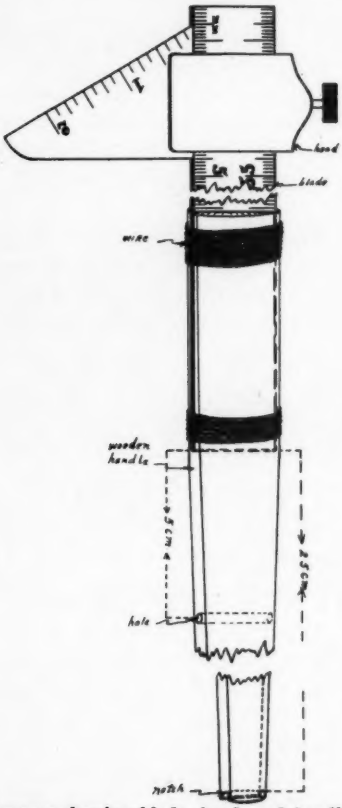

FIG. 1. Drill point gauge showing blade, head, and handle with hole at 5 cm. and notch at 25 cm. from end of steel blade.

gauge consists of a flat, graduated steel blade on which slides a triangular head. The side of the head used in measuring growth extends at right angles from the blade for a distance of about 5 cm. (figure 1).

In actual practice a brass escutcheon nail (number 16 wire, 2 inches long) is driven firmly into the twig (leader in the case of conifers) some distance back from the tip. With this as a base, the distance to the tip of the bud is measured, and after growth starts is remeasured at intervals, the difference between readings giving the amount of growth.

To facilitate holding the base of the blade on the nail, a strong, slotted, wooden handle is slipped over the end of the blade and wired firmly in place. This handle extends 5 cm. beyond the end of the blade and ends in a small notch, which prevents the gauge from slipping off while the gauge head is being moved until it touches the tip of the bud.

If the twig grows vigorously during the season it may grow beyond the length of the blade, in which case a handle 25 cm. or more long may be substituted for the shorter one. There is no difficulty in interpreting the readings when the handles are changed. If some twigs are long and others short, the long handle may be used but with a hole bored 5 cm. from the end of the steel blade to admit the base nail (figure 1).

The drill point gauge as described is made by the L. S. Starrett Company, of Athol, Mass., with heads of two sizes, large and small, and is provided with several lengths of blades, all of which, however, are too short or are provided with unsuitable graduations. However, the 60 cm. blade from another standard tool known as a combination square fits the large-size head perfectly. This blade is graduated in millimeters and half millimeters. It might be advantageous to use a meter blade but such a length is not available as a regular stock tool. Since a wooden handle notched to rest on the base nail is desirable, and since these handles can easily be made in various lengths, the shorter blade is not a serious handicap. The gauge as described can be easily and quickly obtained through local dealers at a cost of about $6.00.

For foresters desiring measurements in 10ths of inches a special blade would have to be made, as regular stock blades in inches are graduated only in 32nds and 64ths.

The writer has used this gauge in making thousands of measurements on the leader growth of various conifers and has found it rapid and yet accurate. With a solid base to measure from and a sharp pointed bud to measure to, growth of as little as a millimeter per day can be accurately determined, provided the set screw is adjusted so that the head slides on the blade without "play." One possible improvement might be the insertion of a tension spring to hold the head from slipping too easily and yet take

up the "play" without undue adjustment of the set screw. This gauge should also be of value in measuring height growth in herbaceous plants, using the ground as a firm base from which to measure.

In measuring the thickness of twigs, a small steel caliper rule (75 mm. long) graduated in half millimeters, also a standard stock tool, was found indispensable for accuracy and speed.—RAYMOND KIENHOLZ, *Yale School of Forestry and Connecticut Agricultural Experiment Station, New Haven, Conn.*

NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists has resulted in the election of the following officers:

President: Dr. D. R. Hoagland, University of California;
Vice-President: Dr. C. O. Appleman, University of Maryland;
Secretary-Treasurer: Dr. W. A. Gardner, Alabama Polytechnic Institute.

A very busy season usually confronts the newly elected officers, as there is always an approaching annual meeting, with its many problems and difficulties. The officers will appreciate cooperation on the part of the members of committees, and of the members generally. The prompt performance of each duty as it comes along makes a pleasure of service.

International Botanical Congress.—According to the decision of the Fifth International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive Committee has been formed, President of which is Professor Doctor F. A. F. C. Went (Utrecht). Professor Doctor J. C. Schoute (Groningen) will act as Vice-President, Dr. W. C. deLeeuw (Bilthoven) as Treasurer, and Dr. M. J. Sirks (Wageningen) as Secretary. The Committee has decided that the VI Congress will meet at Amsterdam, September 9th to 14th, 1935. Scientific societies are kindly requested to reckon with these dates in planning their own meetings.

Osborne Medal.—The American Association of Cereal Chemists has awarded its Thomas Burr Osborne gold medal to Dr. Clyde H. Bailey, Professor of Agricultural Biochemistry at the University of Minnesota, for distinguished contributions in cereal chemistry. Formal presentation of the medal was made at the annual meeting of the Association at Detroit, May 23–26, 1932. Dr. Bailey has been very active in his chosen field and deserves the honor which has been bestowed upon him. He is a member of the American Society of Plant Physiologists, and gave much helpful advice to the officers of the Society at the Kansas City meeting in 1925, when the plans for the establishment of Plant Physiology as our official journal were under discussion.

George Janssen.—News of the death of Dr. George Janssen of the University of Arkansas at Fayetteville, a member of the American Society of Plant Physiologists, came as a distinct shock to those who had just re-

cently met him at the meeting of the Society in New Orleans. A sad commentary on the ways of life is provided by the fact that he there served on the Resolutions Committee. A seemingly indefatigable worker, JANSSEN had not been endowed with the best of health, which accounts for his relatively sudden demise at the age of 35 years.

Following his early years spent in South Dakota, JANSSEN obtained his collegiate training at the State College of that commonwealth and received the B.S. degree from that institution in 1921. From 1920 to 1922 he served as Agent of the Cereal Office, United States Department of Agriculture, and in the latter year was granted the M.S. degree for work in agronomy. During this period he became co-author of experiment station bulletins on the culture of potatoes and wheat.

From 1923 to 1926 JANSSEN served as Research Assistant at the University of Wisconsin, in cooperation with the Office of Cereal Investigations of the U. S. D. A. In 1925 he was awarded the degree of Ph.D. at Wisconsin. His thesis, dealing with the relation of seeding date to the physiology and winter-hardiness of wheat, appeared in the Journal of the American Society of Agronomy **21**: 168–200, 444–466. 1929. Since 1926 Dr. JANSSEN had held the position of Assistant Professor of Agronomy at the University of Arkansas. There he had participated in some of the newer phases of work on the functions of potassium in plant nutrition.

To the professional colleagues of Dr. GEORGE JANSSEN the Society expresses its regret for the untimely loss of an enthusiastic member; and to Mrs. JANSSEN, an alumna of South Dakota State College, and the two children, it extends sympathy.—W. E. T.

Endowment.—Every scientific organization which assumes as part of its services the support of publication of research needs a back-log of permanent income from endowments. The reason for this need is especially obvious in times of stress like the present, when the less active and non-productive support is lost. It is recognized that the best investigators in such an organization are its best assets, for it is the research of such individuals that supplies the commodity for which we are willing to pay. But as it costs on the average of $5.00 for each printed page, a single important paper may cost more than the individual investigator will pay in the form of dues during his entire life. The answer to the problem presented is endowment. The American Society of Plant Physiologists has recognized the need of a strong financial basis for its work. Two of its three endowment funds are fairly well established. There should be a much larger general endowment fund. As a first objective in building such a fund we suggest that $25,000 would provide income equal to 25 per cent.

of the present cost of publication. If this amount were available now, it would not be necessary to delay publication of papers because of lack of space. Everything else about research is endowed except publication. As research becomes useful only upon dissemination and assimilation into the knowledge, culture, and the practices of civilization, the publication of research is the final necessary step before such assimilation can take place. Why should we not have a named endowment for support of publication, if we have named endowments for research itself? This phase of support has been neglected by men of wealth. There is an opportunity for some man of far vision to make an outstanding contribution to research, by establishing endowments to assist in publication under conditions which insure use of the interest solely for meritorious publication.

Minnesota Section.—During the year 1931–1932 the University of Minnesota Section has held eight meetings. The program of addresses before the group was as follows:

Nov. 10, 1931. (a) Physiological relations between stock and scion. Mr. ERNEST ANGELO. (b) Portraits of plant physiologists. Dr. R. B. HARVEY.

Dec. 1. (a) Plant physiology of the vitamins. Dr. G. O. BURR. (b) Physiology of the blueberry. Mr. F. B. CHANDLER.

Jan. 12, 1932. Transformations of iron in the soil. Mr. H. O. HALVORSON.

Feb. 2. Accumulation of dry matter in corn leaves. Mr. K. H. BURNETT, Concordia College.

March 1. Physiological studies of soil algae. Dr. C. E. SKINNER.

April 5. The physiological development of the female gametophyte. Dr. F. K. BUTTERS.

May 3. Relation of seasonal variations to the eradication of weeds. Prof. A. C. ARNY.

June 7. Time of harvest in relation to yield and quality of the crop. Dr. H. K. WILSON.

Purdue University Section.—The Purdue University Section of the American Society of Plant Physiologists held its annual spring dinner meeting on Monday evening May 9, 1932. Guests of the occasion were Dr. H. E. HAYWARD and Dr. E. J. KRAUS of the University of Chicago, Dr. S. H. ECKERSON of the Boyce Thompson Institute, Dr. G. T. NIGHTINGALE of the New Jersey Agricultural Experiment Station and J. P. MARTIN of the Hawaiian Sugar Planters' Experiment Station. Dr. HAYWARD gave a talk on "Some Botanical Problems" in which he reviewed briefly the history of plant morphology, emphasized present deficiencies in our knowledge of ontogeny, and warned against the modern tendency to excessive and pre-

mature publications. On the following day the visitors inspected the research work in the plant sciences at Purdue.

Introduction to Phytochemical Research.—A text-book in mimeographed form bearing this title has been prepared by Dr. R. C. BURRELL, of the Ohio State University, Columbus, Ohio. It is especially designed for advanced students in agricultural chemistry, botany, farm crops, horticulture, soils, etc. Although it was prepared especially for his own classes, Dr. Burrell states that a few copies are available, in case students and investigators in other institutions would like to obtain a copy. The price of the volume is $2.00, and Dr. BURRELL will supply it to any one who desires a copy at that price until the edition is exhausted.

Methods of Analysis.—The third edition of this valuable work was issued by the Association of Official Agricultural Chemists some months ago. A number of new chapters have been added, which give methods of analysis of caustic poisons, naval stores, paints, radioactive substances, and eggs and egg products. Other changes are noted as follows: The chapter on vinegar is now included in the chapter on condiments and spices. Gelatin is included under meat. Beers, wines and distilled liquors have all been combined in one chapter. Feeding stuffs are now included as grain and stock feeds. The appendix defines certain fertilizer terms as adopted by the association. The price of the new edition is $5.00 per volume, with 20 per cent. discount in lots of five or more.

Handbook of Plant Analysis.—The Vienna press of Julius Springer has just issued the second volume of G. KLEIN'S Handbuch der Pflanzenanalyze, a work of 973 pages. It is divided into two sections, the first of which (pp. 1–204) is devoted to the analysis of inorganic materials, and the second (pp. 205–973), to organic substances.

The inorganic substances are considered in four sections: analysis of the most important cations (K, Na, Ca, Mg, Mn, Fe, Al), and anions (CO_3, Cl, I, Br, P_2O_5, SO_3, SiO_2), and of occasionally occurring ions (As, Cu, Zn, Co, Ni); analysis of inorganic nitrogen compounds; determination and analysis of ash; and gas and micro-gas analysis.

The organic substances are treated in thirteen sections with the following titles: alcohols; aldehydes and ketones; phenols and quinons; the organic acids; phosphoric acid esters of carbohydrate metabolism; lipoids; fats and waxes; polyhydric (sugar) alcohols; simple carbohydrates (mono- and disaccharides); characteristics, determination and preparation of the individual sugars; and polysaccharides (inulin, glycogen, starch). The index of the volume occupies 85 pages (889–973).

It is a very valuable handbook, and will prove useful in the selection of methods to be used in physiological problems, the solution of which requires the analysis of plant tissues. The price quoted by the publisher for this paper bound volume is RM 96; with cloth binding, RM 99. Orders should be sent to Julius Springer, Vienna I, Schottengasse 4.

Oils and Fats.—During the last two years a second edition of Ubbelohde's Handbuch der Chemie und Technologie der Öle und Fette has been in process of publication. The editor of this important work is HANS HELLER, of Magdeburg. The first, third, and fourth volumes appeared ahead of volume II. The first part of volume II, a book of 824 pages, has now been published with the title Chemie und Technologie der pflanzlichen Öle and Fette. The work is organized on the basis of the kind of oils occurring in plants. The first section (pp. 1–164) surveys the strong drying oils; the second section (pp. 165–382), the weak drying oils; section three (pp. 383–532), the non-drying oils; and the final section (pp. 533–750), the solid fats. In addition there is a brief section on oil cakes, a supplement, and author and subject indexes.

Within each of the main sections, the oils are arranged in the order of the plant families in which they occur, the system of ENGLER and PRANTL being used. This makes it easy to refer to the oils occurring in any family of plants which may be under investigation.

The work is a great storehouse of information, and will be useful to physiologists as well as technological workers with the oils and fats. Orders should be sent to S. Hirzel, Leipzig C 1. The price is quoted as RM 73 for brochure, and RM 79 for a cloth bound copy of this volume on the plant oils and fats.

Soil Conditions and Plant Growth.—The sixth edition of Sir JOHN RUSSELL's famous monograph has been published by Longmans, Green & Co. It has been thoroughly revised, only the introduction remaining practically unchanged. The order of treatment follows that of the preceding edition, but several of the chapters have slightly changed titles. The book has increased to 636 pages. The fact that the first edition in 1912 was a modest volume of 168 pages shows how the subject has grown in 20 years. This book is the best work of its kind in the English language and has been deservedly popular since its first appearance. No one is more masterful in presenting the relations of soils to plant growth than the gifted author of this work. The price of the new volume is 21 shillings.